Dkt. 59338-A

Application
for
United States Letters Patent

To all whom it may concern:

Be it known that we,

Beth E. Borowsky, Kristine L. Ogozalek. and Kenneth A. Jones

have invented certain new and useful improvements in

DNA Encoding SNORF33 Receptor

of which the following is a full, clear and exact description.

Applicants: Beth E. Borowsky, et al.
U.S. Serial No.: 09/980,145
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Exhibit 9

DNA ENCODING SNORF33 RECEPTOR

BACKGROUND OF THE INVENTION

This application is a continuation-in-part of U.S. Serial No. 09/322,257, filed May 28, 1999, the contents of which are hereby incorporated by reference into the subject application.

Throughout this application various publications are referred to by partial citations within parentheses. Full citations for these publications may be found at the end of the specification immediately preceding the claims. The disclosures of these publications, in their entireties, are hereby incorporated by reference into this application in order to describe more fully the state of the art to which the invention pertains.

Neuroregulators comprise a diverse group of natural products that subverse or modulate communication in the nervous system. They include, but are not limited to, neuropeptides, amino acids, biogenic amines, lipids, and lipid metabolites, and other metabolic byproducts. Many of these neuroregulator substances interact with specific cell surface receptors, which transduce signals from the outside to the inside of the cell. G-protein coupled receptors (GPCRs) represent a major class of cell surface receptors with which many neurotransmitters interact to mediate their effects. GPCRs are characterized by seven membrane-spanning domains and are coupled to their effectors via G-proteins linking receptor activation with intracellular biochemical sequelae such as stimulation of adenylyl cyclase.

Tyramine (TYR), β-phenyl-ethylamine (PEA), tryptamine (T) and octopamine (OCT) belong to a class of amines that have low endogenous levels in tissues and thus are referred to as "trace amines" (Usdin and Sandler, 1976). For example, under

physiological conditions, brain levels of T in the rat are a thousand-fold lower than those of 5-hydroxytryptamine (5-HT), a major neurotransmitter in vertebrates and invertebrates (Artigas and Gelpi, 1979).

In invertebrates, the role of "trace amines" as neurotransmitters is well established, in particular for OCT and TYR, whose physiological actions have been shown to be mediated directly via their specific receptors. Octopamine, the monohydroxylated analogue of NE, has been studied the most in this respect and is a major neurotransmitter, neurohormone and neuromodulator in many invertebrate species (Axelrod and Saavedra, 1977; David and Coulon, 1985). Because many of the octopamine-mediated responses are connected to adaptation to stressful circumstances, the octopaminergic system has been considered to be the invertebrate equivalent of the vertebrate sympathetic nervous system. Recently, the cloning of the first invertebrate (mollusc) OCT receptor has been reported and it belongs to the family of G protein coupled receptors (GPCR) (Gerhardt et al., 1997). Similarly, TYR which is the precursor of OCT, is abundant in insect brains and its distribution in different tissues does not parallel that of OCT, suggesting that TYR is not merely a precursor in the biosynthetic pathway of OCT (Juorio and Sloley, 1988; Maxwell et al., 1978). In fact, TYR and OCT have opposite effects on adenylate cyclase and glycogenolysis in cockroach fat bodies, TYR being inhibitory and OCT being stimulatory (Downer, 1979). Therefore, in addition to OCT, TYR has also been suggested to play a role as a neurotransmitter in invertebrates (Roeder, 1994). Cloning of an adenylate cyclase inhibitory Drosophila TYR receptor, belonging to the family of GPCRs (Saudou et al., 1990) supports this hypothesis.

The evidence for the role of "trace amines" as neurotransmitters in the mammalian system has not been carefully studied. Because of the low concentrations (<100ng/g) of "trace amines" in

mammalian tissues it has sometimes been suggested that they might occur as by-products in the synthesis of other amine neurotransmitters such as the catecholamines or 5-HT. It is now apparent that the turnover of the "trace amines" in most tissues is very rapid, as evidenced by their loss from the brain after intraventricular administration (Wu and Boulton, 1973) and by their accumulation after inhibition of their major catabolic enzyme, monoamine oxidase (MAO) (Axelrod and Saavedra, 1974; Juorio and Durden, 1984; Philips and Boulton, 1979). Due to the fact that these "trace amines" share synthetic and catabolic enzymes with the classical amines, such as 5-HT, norepinephrine (NE) and dopamine (DA), they have also been referred to as "false transmitters" (McGeer et al., 1979). These amines are thus taken up by aminergic neurons, displace monoamines from their storage sites in vesicles, and can themselves and/or other amine neurotransmitters, then be released from neurons by electrical stimulation. Therefore, in mammals, some of the physiological actions of these "trace amines" (sympathomimetic in general, pressor, cardiac stimulant and vasoconstrictor activity) are primarily indirect and are caused by a release of endogenous neurotransmitters (NE, 5-HT, DA).

However, there is a growing body of evidence suggesting that "trace amines" function independently of the classical amine neurotransmitters and mediate some of their effects via their specific receptors. Some of these are described below.

Tyramine is among the first "trace amines" subjected to experimental study. Radiolabeled TYR can be released from rat striatal slices following KCl^- depolarization. In reserpine pretreated rats, TYR induced a marked increase in the motor activity, which was not accompanied by a significant decrease in brain catecholamines, ruling out the possibility of indirect receptor stimulation (Stoof et al., 1976). A direct endothelium-

and β2-adrenoceptor independent vasorelaxant effect of TYR on rat aortic strips has been reported (Varma and Chemtob, 1993; Varma et al., 1995). Saturable binding sites for [^{3}H]*p*-tyramine have been reported in rat brain, which may reflect specific TYR receptor sites (Ungar et al., 1977; Vaccaria, 1986; Vaccaria, 1988). Further studies are needed before a clear definition of specifc *p*-tyramine binding site is available. There are no reports of *m*-tyramine binding sites available as yet.

β-Phenylethylamine which has a chemical structure and pharmacological and behavioral effects that closely resemble those of amphetamine (evokes stereotyped behavior, anorexia and increases locomotor activity) (Wolf and Mosnaim, 1983) and has been described as the body's natural amphetamine. β-Phenylethylamine is synthesized in and released by dopaminergic neurons of the nigrostriatal system (Greenshaw et al., 1986). Saturable, high affinity binding sites have been reported for [^{3}H]β-Phenylethylamine (Hauger et al., 1982). The highest concentration of binding sites was in the hypothalamus, where highest endogenous levels of this amine has been reported (Durden and Philips, 1973). Interestingly, saturable binding sites have also been reported for [^{3}H]amphetamine in membrane preparations from rat brain (Paul et al., 1982), the density of these binding sites being highest in the hypothalamus, as has been seen with PEA binding. These binding sites were shown not to be associated with any previously described neurotransmitter or drug receptor sites and were specific to amphetamine and related PEA derivatives. Furthermore, the relative affinities of a series of PEA derivatives for this binding site were highly correlated to their potencies as anorexic agents. These results suggest the presence of specific receptor sites in the hypothalamus that mediate the anorexic activity of amphetamine and related PEAs.

In addition to TYR and PEA, T has also been shown to produce

several physiological effects that are direct and distinct from those mediated by other aminergic neurotransmitters. Tryptamine has been shown to have opposite effects to 5-HT in several systems studied. For example, unilateral intrahypothalamic injection of T into the preoptic area of the rat causes hyperthermia, whereas 5-HT administered into the same area produces the opposite effect (Cox et al., 1981; Cox et al., 1983). Intravenous administration of T to young rats leads to behavioral stimulation and electrocortical desynchronization, whereas behavioral depression and electrical synchronization was evoked by 5-HT (Dewhurst and Marley, 1965). Also, iontophoretic application of 5-HT and T to cortical neurons has been noted to produce excitatory and inhibitory responses, respectively (Jones, 1982b,c). Injection of deuterated T into the nucleus accumbens of the rat produces sustained locomotor stimulation (Marien et al., 1987), whereas 5-HT injection into the same area produces either only a transient decrease in locomotor activity (Pijnenburg et al., 1976) or no significant effect on locomotion (Gerber et al., 1986; Jackson et al., 1975; Kitada et al., 1983; Plaznik et al., 1985). Tryptamine as well as 5-HT cause contraction of the rat stomach fundus. However, using the non-selective antagonist, phenoxybenzamine (PBZ), Winter and Gessner (1968) showed that the T-induced contractions were more resistant to PBZ blockade than 5-HT-induced contractions. Also, tetrahydro-β-carboline (THBC) antagonizes tryptamine, but not 5-HT-mediated contraction of the isolated rat tail artery (Hicks and Langer, 1983).

The presence of specific, saturable and high affinity $[^3H]$-T binding sites in the rat brain (Altar et al., 1986; Kellar and Cascio, 1982; McCormack et al., 1986; Perry, 1986) and peripheral tissue (Brüning and Rommelspacher, 1984) has now been known for a few years. The pharmacological profile of $[^3H]$-T binding is distinct and does not correspond to any known neurotransmitter,

transporter or MAO site (Biegon et al., 1982; Fuxe et al., 1983; Leysen et al., 1982; Meibach et al., 1980, 1982; Nakada et al., 1984; Palacios et al., 1983; Perry, 1986, 1988; Slater and Patel, 1983).

The existence of *p*-octopamine binding sites has been demonstrated in crude membranes obtained from fruitflies but not shown so far in vertebrates (Dudai, 1982; Dudai and Zvi, 1984; Hashemzadeh et al., 1985).

The above findings indicate that in the mammalian system, TYR, PEA and T may function as neurotransmitters in their own rights, and mediate their responses via acting at their distinctive receptors.

"Trace amines" could play a role in depression and psychiatric disorders as well as migraine. Clinical literature supports these indications. MAO inhibitors that are clinically effective for the treatment of depression in the human, have been shown to produce a proportionally greater increase in "trace amine" levels compared to 5-HT levels (Boulton, 1976; Juorio, 1976). Based on a functional deficiency of "trace amines", PEA and/or T in particular, have been proposed as a possible etiological factor in depression in humans (Dewhurst, 1968a, b; Dewhurst and Marley, 1965; Sabelli and Monsnaim, 1974). The urinary output of T has been shown to be disturbed in schizophrenic patients (Brune and Himwhich, 1962; Herkert and Keup, 1969) and in the general psychiatric population (Slingsby and Boulton, 1976). The urinary output of T seems to be positively correlated with increasing severity of psychosis (Brune and Himwhich, 1962; Herkert and Keup, 1969). Depressed patients on the other hand, exhibit decreased urinary output of T (Coppen et al., 1965) and OCT (Sandler et al., 1979).

A role of "trace amines" in migraine is implicated, since certain

pharmacological agents in food, in particular TYR, are believed to provoke migraine. There are many reports that attacks of palpitation, hypertension and severe headache (the so called "cheese effect") may follow the consumption of food containing TYR in patients being treated with MAO inhibitors (see Vaughan, 1994 for review). Furthermore, clinical studies have shown that migraine sufferers had lower urinary excretion of TYR sulphate following oral TYR challenge than normal controls. The lower TYR sulfate excretion values among patients with both migraine and depression compared to those of migraine alone or depression alone suggest that comorbid migraine with depression may represent a more severe form of migraine than migraine alone (Merikangas et al., 1995). It is likely that disturbances in the same neurochemical systems, most probably the "trace amines", account for the co-occurrence of migraine and depression.

Urinary levels of PEA, TYR and indole-3-acetic acid (the acid metabolite of T) were found to be decreased in Tourette's Syndrome (TS) patients when compared to values in normal children, indicating a role of these "trace amines" in TS (Baker et al., 1993). Urinary levels of PEA have been shown to be significantly lower in patients with learning disability (LD) and in patients suffering from Attention Deficit Hyperactivity Disorder (ADHD) as compared to age-matched controls, indicating an important role of PEA in pathogenesis of LD and ADHD (Matsuishi and Yamashita, 1999). Tryptamine has also been implicated to play a role in Parkinson's disease, since Parkinsonian patients excrete abnormally high levels of T in their urine (Smith and Kellow, 1969).

Altered "trace amine" metabolism has been observed in non-psychiatric conditions such as pellagra (Sullivan, 1922), Hartnup's disease (Baron et al., 1956), phenylketonuria (Armstrong and Robinson, 1954; Perry, 1962) and thyrotoxicosis (Levine et al., 1962).

Studies in non-human species, rats and mice in particular, add further support for some of the roles of the "trace amines" described above as well as providing various additional physiological roles of "trace amines", as discussed below.

Interestingly, MAO A knock-out mice have elevated brain levels of 5-HT, NE and DA and manifest aggressive behavior similar to human males with a deletion of MAO A. In contrast, MAO B knock-out mice do not exhibit aggression and only levels of PEA are increased. Mice lacking MAO B are resistant to the Parkinsongenic neurotoxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPP^{+}) (Shih et al., 1999), indicating that PEA may provide neuroprotection. Both MAO A and MAO B knock-out mice show increased reactivity to stress suggesting a role of PEA in this condition.

A possible role for T in the protection against renal hypertension afforded by TRP has been suggested (Fregly et al., 1988). A role of OCT in hypertension has been suggested since a hypertensive strain of rats (SHR Kyoto) demonstrates considerably elevated levels of this amine in their brain (David, 1978; 1979). Housing stress has been shown in rats to cause an increase brain and adrenal T levels (Harrison and Christian, 1984) which may be the cause of cardiovascular changes (Bennett and Gardiner, 1978) and hyperactivity (Weinstock et al., 1978) observed in these animals. Therefore, T has been proposed to play a role in the physiological, behavioral and chemical response to psychological stress.

Tryptamine's actions in the stomach and the presence of [^{3}H]-T binding sites in the stomach suggest a role for T in gastric emptying and control of secretory processes (Brüning and Rommelspacher, 1984; Cohen and Wittenauer, 1985; Winter and Gessner, 1968).

Tryptamine has also been suggested to play a role in hepatic encephalopathy where, due to liver failure, there is a massive increase in brain TRP (precursor of T) leading to a series of CNS symptoms including altered sleep patterns and personality changes and eventually resulting in coma (Sourkes, 1978).

Tryptamine has been shown to cause release from isolated rat lungs of a spasmogen, resembling slow reacting substance of anaphylaxis that has prostaglandin E-like activity (Bakhle and Smith, 1977). Therefore, T may have a function in asthma.

In summary, "trace amines" may act as neurotransmitters and neuromodulators. These amines may act via their specific receptor sites to elicit some of their physiological actions. It is not yet clear what the role of these "trace amines" is in pathological conditions such as mental and physical stress, hepatic dysfunction, hypertension and electrolyte imbalance. A primary role of "trace amines" in the etiology of mental or neurological diseases is still hypothetical. "Trace amine" - mediated effects indicate that receptors for these amines are attractive as targets for therapeutic intervention for several disorders and would be useful to develop drugs with higher specificity and fewer side effects for a wide variety of diseases.

SUMMARY OF THE INVENTION

This invention provides an isolated nucleic acid encoding a mammalian SNORF33 receptor.

This invention further provides a purified mammalian SNORF33 receptor protein.

This invention also provides a vector comprising a nucleic acid in accordance with this invention.

This invention still further provides a cell comprising a vector in accordance with this invention.

This invention additionally provides a membrane preparation isolated from a cell in accordance with this invention.

Furthermore, this invention provides a nucleic acid probe comprising at least 15 nucleotides, which probe specifically hybridizes with a nucleic acid encoding a mammalian SNORF33 receptor, wherein the probe has a sequence complementary to a unique sequence present within one of the two strands of the nucleic acid encoding the mammalian SNORF33 receptor contained in plasmid pcDNA3.1-hSNORF33-f (Patent Deposit Designation PTA-398), plasmid pcDNA3.1-rSNORF33-f (Patent Deposit Designation PTA-102) or plasmid pEXJ-hSNORF33-f (Patent Deposit Designation PTA-570).

This invention further provides a nucleic acid probe comprising at least 15 nucleotides, which probe specifically hybridizes with a nucleic acid encoding a mammalian SNORF33 receptor, wherein the probe has a sequence complementary to a unique sequence present within (a) the nucleic acid sequence shown in Figures 5A-5B (SEQ ID NO: 5) or (b) the reverse complement thereof.

This invention provides an antisense oligonucleotide having a sequence capable of specifically hybridizing to RNA encoding a mammalian SNORF33 receptor, so as to prevent translation of such RNA.

This invention further provides an antisense oligonucleotide having a sequence capable of specifically hybridizing to genomic DNA encoding a mammalian SNORF33 receptor, so as to prevent transcription of such genomic DNA.

This invention also provides an antibody capable of binding to a mammalian SNORF33 receptor encoded by a nucleic acid in accordance with this invention.

Moreover, this invention provides an agent capable of competitively inhibiting the binding of an antibody in accordance with this invention to a mammalian SNORF33 receptor.

This invention yet further provides a pharmaceutical composition comprising (a) an amount of an oligonucleotide in accordance with this invention capable of passing through a cell membrane and effective to reduce expression of a mammalian SNORF33 receptor and (b) a pharmaceutically acceptable carrier capable of passing through the cell membrane.

This invention also provides a pharmaceutical composition which comprises an amount of an antibody in accordance with this invention effective to block binding of a ligand to a human SNORF33 receptor and a pharmaceutically acceptable carrier.

This invention further provides a transgenic, nonhuman mammal expressing DNA encoding a mammalian SNORF33 receptor in accordance with this invention.

This invention still further provides a transgenic, nonhuman

mammal comprising a homologous recombination knockout of a native mammalian SNORF33 receptor.

This invention further provides a transgenic, nonhuman mammal whose genome comprises antisense DNA complementary to DNA encoding a mammalian SNORF33 receptor in accordance with this invention so placed within such genome as to be transcribed into antisense mRNA which is complementary to and hybridizes with mRNA encoding the mammalian SNORF33 receptor so as to reduce translation of such mRNA and expression of such receptor.

This invention provides a process for identifying a chemical compound which specifically binds to a mammalian SNORF33 receptor which comprises contacting cells containing DNA encoding, and expressing on their cell surface, the mammalian SNORF33 receptor, wherein such cells do not normally express the mammalian SNORF33 receptor, with the compound under conditions suitable for binding, and detecting specific binding of the chemical compound to the mammalian SNORF33 receptor.

This invention further provides a process for identifying a chemical compound which specifically binds to a mammalian SNORF33 receptor which comprises contacting a membrane preparation from cells containing DNA encoding, and expressing on their cell surface, the mammalian SNORF33 receptor, wherein such cells do not normally express the mammalian SNORF33 receptor, with the compound under conditions suitable for binding, and detecting specific binding of the chemical compound to the mammalian SNORF33 receptor.

This invention still further provides a process involving competitive binding for identifying a chemical compound which specifically binds to a mammalian SNORF33 receptor which comprises separately contacting cells expressing on their cell surface the mammalian SNORF33 receptor, wherein such cells do not

normally express the mammalian SNORF33 receptor, with both the chemical compound and a second chemical compound known to bind to the receptor, and with only the second chemical compound, under conditions suitable for binding of such compounds to the receptor, and detecting specific binding of the chemical compound to the mammalian SNORF33 receptor, a decrease in the binding of the second chemical compound to the mammalian SNORF33 receptor in the presence of the chemical compound being tested indicating that such chemical compound binds to the mammalian SNORF33 receptor.

This invention further provides a process involving competitive binding for identifying a chemical compound which specifically binds to a mammalian SNORF33 receptor which comprises separately contacting a membrane preparation from cells expressing on their cell surface the mammalian SNORF33 receptor, wherein such cells do not normally express the mammalian SNORF33 receptor, with both the chemical compound and a second chemical compound known to bind to the receptor, and with only the second chemical compound, under conditions suitable for binding of such compounds to the receptor, and detecting specific binding of the chemical compound to the mammalian SNORF33 receptor, a decrease in the binding of the second chemical compound to the mammalian SNORF33 receptor in the presence of the chemical compound being tested indicating that such chemical compound binds to the mammalian SNORF33 receptor.

This invention further provides a compound identified by one of the processes of this invention.

This invention provides a method of screening a plurality of chemical compounds not known to bind to a mammalian SNORF33 receptor to identify a compound which specifically binds to the mammalian SNORF33 receptor, which comprises (a) contacting cells transfected with, and expressing, DNA encoding the mammalian

SNORF33 receptor with a compound known to bind specifically to the mammalian SNORF33 receptor; (b) contacting the cells of step (a) with the plurality of compounds not known to bind specifically to the mammalian SNORF33 receptor, under conditions permitting binding of compounds known to bind to the mammalian SNORF33 receptor; (c) determining whether the binding of the compound known to bind to the mammalian SNORF33 receptor is reduced in the presence of the plurality of compounds, relative to the binding of the compound in the absence of the plurality of compounds; and if so (d) separately determining the binding to the mammalian SNORF33 receptor of each compound included in the plurality of compounds, so as to thereby identify any compound included therein which specifically binds to the mammalian SNORF33 receptor.

This invention further provides a method of screening a plurality of chemical compounds not known to bind to a mammalian SNORF33 receptor to identify a compound which specifically binds to the mammalian SNORF33 receptor, which comprises (a) contacting a membrane preparation from cells transfected with, and expressing, DNA encoding the mammalian SNORF33 receptor with the plurality of compounds not known to bind specifically to the mammalian SNORF33 receptor under conditions permitting binding of compounds known to bind to the mammalian SNORF33 receptor; (b) determining whether the binding of a compound known to bind to the mammalian SNORF33 receptor is reduced in the presence of the plurality of compounds, relative to the binding of the compound in the absence of the plurality of compounds; and if so (c) separately determining the binding to the mammalian SNORF33 receptor of each compound included in the plurality of compounds, so as to thereby identify any compound included therein which specifically binds to the mammalian SNORF33 receptor.

This invention also provides a method of detecting expression of a mammalian SNORF33 receptor by detecting the presence of mRNA

coding for the mammalian SNORF33 receptor which comprises obtaining total mRNA from the cell and contacting the mRNA so obtained with a nucleic acid probe according to this invention under hybridizing conditions, detecting the presence of mRNA hybridized to the probe, and thereby detecting the expression of the mammalian SNORF33 receptor by the cell.

This invention further provides a method of detecting the presence of a mammalian SNORF33 receptor on the surface of a cell which comprises contacting the cell with an antibody according to this invention under conditions permitting binding of the antibody to the receptor, detecting the presence of the antibody bound to the cell, and thereby detecting the presence of the mammalian SNORF33 receptor on the surface of the cell.

This invention still further provides a method of determining the physiological effects of varying levels of activity of mammalian SNORF33 receptors which comprises producing a transgenic, nonhuman mammal in accordance with this invention whose levels of mammalian SNORF33 receptor activity are varied by use of an inducible promoter which regulates mammalian SNORF33 receptor expression.

This invention additionally provides a method of determining the physiological effects of varying levels of activity of mammalian SNORF33 receptors which comprises producing a panel of transgenic, nonhuman mammals in accordance with this invention each expressing a different amount of mammalian SNORF33 receptor.

Moreover, this invention provides a method for identifying an antagonist capable of alleviating an abnormality wherein the abnormality is alleviated by decreasing the activity of a mammalian SNORF33 receptor comprising administering a compound to a transgenic, nonhuman mammal according to this invention, and determining whether the compound alleviates any physiological

and/or behavioral abnormality displayed by the transgenic, nonhuman mammal as a result of overactivity of a mammalian SNORF33 receptor, the alleviation of such an abnormality identifying the compound as an antagonist.

This invention also provides an antagonist identified by the preceding method.

This invention further provides a composition, e.g. a pharmaceutical composition, comprising an antagonist according to this invention and a carrier, e.g. a pharmaceutically acceptable carrier.

This invention additionally provides a method of treating an abnormality in a subject wherein the abnormality is alleviated by decreasing the activity of a mammalian SNORF33 receptor which comprises administering to the subject an effective amount of the pharmaceutical composition according to this invention so as to thereby treat the abnormality.

In addition, this invention provides a method for identifying an agonist capable of alleviating an abnormality in a subject wherein the abnormality is alleviated by increasing the activity of a mammalian SNORF33 receptor comprising administering a compound to a transgenic, nonhuman mammal according to this invention, and determining whether the compound alleviates any physionlogical and/or behavioral abnormality displayed by the transgenic, nonhuman mammal, the alleviation of such an abnormality identifying the compound as an agonist.

This invention further provides an agonist identified by the preceding method.

This invention still further provides a composition, e.g. a pharmaceutical composition, comprising an agonist according to

this invention and a carrier, e.g. a pharmaceutically acceptable carrier.

Moreover, this invention provides a method of treating an abnormality in a subject wherein the abnormality is alleviated by increasing the activity of a mammalian SNORF33 receptor which comprises administering to the subject an effective amount of the pharmaceutical composition according to this invention so as to thereby treat the abnormality.

Yet further, this invention provides a method for diagnosing a predisposition to a disorder associated with the activity of a specific mammalian allele which comprises: (a) obtaining DNA of subjects suffering from the disorder; (b)performing a restriction digest of the DNA with a panel of restriction enzymes; (c) electrophoretically separating the resulting DNA fragments on a sizing gel; (d) contacting the resulting gel with a nucleic acid probe capable of specifically hybridizing with a unique sequence included within the sequence of a nucleic acid molecule encoding a mammalian SNORF33 receptor and labeled with a detectable marker; (e) detecting labeled bands which have hybridized to the DNA encoding a mammalian SNORF33 receptor to create a unique band pattern specific to the DNA of subjects suffering from the disorder; (f) repeating steps (a)-(e) with DNA obtained for diagnosis from subjects not yet suffering from the disorder; and (g) comparing the unique band pattern specific to the DNA of subjects suffering from the disorder from step (e) with the band pattern from step (f) for subjects not yet suffering from the disorder so as to determine whether the patterns are the same or different and thereby diagnose predisposition to the disorder if the patterns are the same.

This invention also provides a method of preparing a purified mammalian SNORF33 receptor according to the invention which comprises: (a) culturing cells which express the mammalian

SNORF33 receptor; (b) recovering the mammalian SNORF33 receptor from the cells; and (c) purifying the mammalian SNORF33 receptor so recovered.

This invention further provides a method of preparing the purified mammalian SNORF33 receptor according to the invention which comprises: (a) inserting a nucleic acid encoding the mammalian SNORF33 receptor into a suitable expression vector; (b) introducing the resulting vector into a suitable host cell; (c) placing the resulting host cell in suitable conditions permitting the production of the mammalian SNORF33 receptor; (d) recovering the mammalian SNORF33 receptor so produced; and optionally (e) isolating and/or purifying the mammalian SNORF33 receptor so recovered.

Furthermore, this invention provides a process for determining whether a chemical compound is a mammalian SNORF33 receptor agonist which comprises contacting cells transfected with and expressing DNA encoding the mammalian SNORF33 receptor with the compound under conditions permitting the activation of the mammalian SNORF33 receptor, and detecting any increase in mammalian SNORF33 receptor activity, so as to thereby determine whether the compound is a mammalian SNORF33 receptor agonist.

This invention also provides a process for determining whether a chemical compound is a mammalian SNORF33 receptor antagonist which comprises contacting cells transfected with and expressing DNA encoding the mammalian SNORF33 receptor with the compound in the presence of a known mammalian SNORF33 receptor agonist, under conditions permitting the activation of the mammalian SNORF33 receptor, and detecting any decrease in mammalian SNORF33 receptor activity, so as to thereby determine whether the compound is a mammalian SNORF33 receptor antagonist.

This invention still further provides a composition, for example

a pharmaceutical composition, which comprises an amount of a mammalian SNORF33 receptor agonist determined by a process according to this invention effective to increase activity of a mammalian SNORF33 receptor and a carrier, for example, a pharmaceutically acceptable carrier. In one embodiment, the mammalian SNORF33 receptor agonist is not previously known.

Also, this invention provides a composition, for example a pharmaceutical composition, which comprises an amount of a mammalian SNORF33 receptor antagonist determined by a process according to this invention effective to reduce activity of a mammalian SNORF33 receptor and a carrier, for example, a pharmaceutically acceptable carrier.

This invention moreover provides a process for determining whether a chemical compound specifically binds to and activates a mammalian SNORF33 receptor, which comprises contacting cells producing a second messenger response and expressing on their cell surface the mammalian SNORF33 receptor, wherein such cells do not normally express the mammalian SNORF33 receptor, with the chemical compound under conditions suitable for activation of the mammalian SNORF33 receptor, and measuring the second messenger response in the presence and in the absence of the chemical compound, a change, e.g. an increase, in the second messenger response in the presence of the chemical compound indicating that the compound activates the mammalian SNORF33 receptor.

This invention still further provides a process for determining whether a chemical compound specifically binds to and inhibits activation of a mammalian SNORF33 receptor, which comprises separately contacting cells producing a second messenger response and expressing on their cell surface the mammalian SNORF33 receptor, wherein such cells do not normally express the mammalian SNORF33 receptor, with both the chemical compound and a second chemical compound known to activate the mammalian

SNORF33 receptor, and with only the second chemical compound, under conditions suitable for activation of the mammalian SNORF33 receptor, and measuring the second messenger response in the presence of only the second chemical compound and in the presence of both the second chemical compound and the chemical compound, a smaller change, e.g. increase, in the second messenger response in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound indicating that the chemical compound inhibits activation of the mammalian SNORF33 receptor.

Further, this invention provides a compound determined by a process according to the invention and a composition, for example, a pharmaceutical composition, which comprises an amount of a mammalian SNORF33 receptor agonist determined to be such by a process according to the invention, effective to increase activity of the mammalian SNORF33 receptor and a carrier, for example, a pharmaceutically acceptable carrier.

This invention also provides a composition, for example, a pharmaceutical composition, which comprises an amount of a mammalian SNORF33 receptor antagonist determined to be such by a process according to the invention, effective to reduce activity of the mammalian SNORF33 receptor and a carrier, for example, a pharmaceutically acceptable carrier.

This invention yet further provides a method of screening a plurality of chemical compounds not known to activate a mammalian SNORF33 receptor to identify a compound which activates the mammalian SNORF33 receptor which comprises: (a) contacting cells transfected with and expressing the mammalian SNORF33 receptor with the plurality of compounds not known to activate the mammalian SNORF33 receptor, under conditions permitting activation of the mammalian SNORF33 receptor; (b) determining whether the activity of the mammalian SNORF33 receptor is

increased in the presence of one or more of the compounds; and if so (c) separately determining whether the activation of the mammalian SNORF33 receptor is increased by any compound included in the plurality of compounds, so as to thereby identify each compound which activates the mammalian SNORF33 receptor.

This invention provides a method of screening a plurality of chemical compounds not known to inhibit the activation of a mammalian SNORF33 receptor to identify a compound which inhibits the activation of the mammalian SNORF33 receptor, which comprises: (a) contacting cells transfected with and expressing the mammalian SNORF33 receptor with the plurality of compounds in the presence of a known mammalian SNORF33 receptor agonist, under conditions permitting activation of the mammalian SNORF33 receptor; (b) determining whether the extent or amount of activation of the mammalian SNORF33 receptor is reduced in the presence of one or more of the compounds, relative to the extent or amount of activation of the mammalian SNORF33 receptor in the absence of such one or more compounds; and if so (c) separately determining whether each such compound inhibits activation of the mammalian SNORF33 receptor for each compound included in the plurality of compounds, so as to thereby identify any compound included in such plurality of comopunds which inhibits the activation of the mammalian SNORF33 receptor.

This invention also provides a composition, for example a pharmaceutical composition, comprising a compound identified by a method according to this invention in an amount effective to increase mammalian SNORF33 receptor activity and a carrier, for example, a pharmaceutically acceptable carrier.

This invention still further provides a composition, for example a pharmaceutical composition, comprising a compound identified by a method according to this invention in an amount effective to decrease mammalian SNORF33 receptor activity and a carrier,

for example a pharmaceutically acceptable carrier.

Furthermore, this invention provides a method of treating an abnormality in a subject wherein the abnormality is alleviated by increasing the activity of a mammalian SNORF33 receptor which comprises administering to the subject a compound which is a mammalian SNORF33 receptor agonist in an amount effective to treat the abnormality.

This invention additionally provides a method of treating an abnormality in a subject wherein the abnormality is alleviated by decreasing the activity of a mammalian SNORF33 receptor which comprises administering to the subject a compound which is a mammalian SNORF33 receptor antagonist in an amount effective to treat the abnormality.

This invention also provides a process for making a composition of matter which specifically binds to a mammalian SNORF33 receptor which comprises identifying a chemical compound using a process in accordance with this invention and then synthesizing the chemical compound or a novel structural and functional analog or homolog thereof.

This invention further provides a process for preparing a composition, for example, a pharmaceutical composition which comprises admixing a carrier, for example, a pharmaceutically acceptable carrier, and a pharmaceutically effective amount of a chemical compound identified by a process of in accordance with this invention or a novel structural and functional analog or homolog thereof.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1

Nucleotide sequence including part of the sequence encoding a human SNORF33 receptor (SEQ ID NO: 1).

Figure 2

Deduced amino acid sequence (SEQ ID NO: 2) of the human SNORF33 receptor encoded by the nucleotide sequence shown in Figure 1 (SEQ ID NO: 1).

Figures 3A-3B

Nucleotide sequence including sequence encoding a rat SNORF33 receptor (SEQ ID NO: 3). Putative open reading frames including the shortest open reading frame are indicated by underlining one start (ATG) codon (at positions 53-55) and the stop codon (at positions 1049-1051). In addition, partial 5' and 3' untranslated sequences are shown.

Figures 4A-4B

Deduced amino acid sequence (SEQ ID NO: 4) of the rat SNORF33 receptor encoded by the longest open reading frame indicated in the nucleotide sequence shown in Figures 3A-3B (SEQ ID NO: 3). The seven putative transmembrane (TM) regions are underlined.

Figures 5A-5B

Nucleotide sequence including sequence encoding a human SNORF33 receptor (SEQ ID NO: 5). Putative open reading frames including the shortest open reading frame are indicated by underlining two start (ATG) codons (at positions 7-9 and 10-12) and the stop codon (at positions 1024-1026). In addition, partial 5' and 3' untranslated sequences are shown.

Figures 6A-6B

Deduced amino acid sequence (SEQ ID NO: 6) of the human SNORF33 receptor encoded by the longest open reading frame indicated in the nucleotide sequence shown in Figures 5A-5B (SEQ ID NO: 5). The seven putative transmembrane (TM) regions are underlined.

Figures 7A-7B

Allignment of the rat and human SNORF33 amino acid sequences. Conserved residues are indicated by a vertical line and similar residues are indicated by single or double dots. Gaps in the alignment are indicated by dots in the sequence.

Figure 8

Basal cAMP levels in COS-7 cells transfected with DNA vector (Mock)- and rSNORF33 DNA. The data are presented as mean±S.E.M. Number of experiments = 6-7.

Figure 9

Stimulation of intracellular cAMP release by agonists in DNA vector (Mock)- and rSNORF33-transfected COS-7 cells. The data are presented as mean±S.E.M. Number of experiments = 3-8.

Figure 10

Effect of pharmacological agents on intracellular cAMP levels in DNA vector (Mock)- and rSNORF33-transfected COS-7 cells. The data are presented as mean±S.E.M. Number of experiments = 3-8.

Figures 11A and 11B

(Figure 11A) Example of a cumulative concentration-response to octopamine in an oocyte expressing SNORF33 and CFTR. Oocyte was voltage clamped to -80 mV and drug was applied at increasing concentrations as indicated by the horizontal bars.

(Figure 11B) Plot of concentration-response data for tyramine,

tryptamine, octopamine and 5-HT multiple batches of oocytes expressing SNORF33 and CFTR. n = 4-7 oocytes for each data point. Curves were fit using the Hill equation of the form $I = 1 / (1 + (EC50 / [Agonist])^n)$.

Figure 12

Antagonist profile of a variety of compounds tested at single doses (100 μM except where noted) for their ability to inhibit responses to an EC_{80} concentration of tyramine (100 nM) in oocytes expressing SNORF33 and CFTR. Responses in the presence of test compound were normalized to the current stimulated by an application of tyramine applied in the absence of test compound.

Figure 13

Saturation binding of $[^3H]$-TYR. COS-7 cells were transiently-transfected with rSNORF33 and membranes were prepared as described in Materials and Methods. Membranes (40 - 70 μg protein) were incubated at 4° C with increasing concentrations of $[^3H]$-TYR (0.1 nM - 70 nM) for 30 minutes. Non-specific binding was determined in the presence of 10 μM TYR and represented < 10% of total binding. Results are representative of two independent experiments with average Kd = 12.5 nM and Bmax = 1400 fmol/mg protein.

Figure 14

Representative curves for various "trace amines" displacing $[^3H]$-TYR binding to rSNORF33. The indicated compounds were evaluated in competition binding assays on membranes from rSNORF33 using $[^3H]$-TYR (7 - 15 nM) as the radioligand. Non-specific binding was determined in the presence of 10 μM TYR and data were fit to non-linear curves using GraphPad Prism. Ki values were determined using the Cheng-Prussoff correction.

Figure 15

Specific binding of [^{3}H]-T to rSNORF33- and mock-transfected COS-7 cell membranes. Binding assay using 20 nM [^{3}H]-T was performed according to the Methods. The data are presented as mean+S.E.M. of quadruplicate determinations.

Figure 16

Electrophysiological response of an oocyte expressing hSNORF33 and CFTR. Bar indicates the application of 100 μM tyramine. Break in the trace respresents a 5 second gap in the recording. Oocyte was voltage clamped to -80 mV.

Figure 17

Nucleotide sequence including part of the sequence encoding a mouse SNORF33 receptor (SEQ ID NO: 30).

Figure 18

Deduced partial amino acid sequence (SEQ ID NO: 31) of the mouse SNORF33 receptor encoded by the nucleotide sequence shown in Figure 17 (SEQ ID NO: 30). Putative transmembrane (TM) regions are underlined.

DETAILED DESCRIPTION OF THE INVENTION

This invention provides a recombinant nucleic acid comprising a nucleic acid encoding a mammalian SNORF33 receptor, wherein the mammalian receptor-encoding nucleic acid hybridizes under high stringency conditions to (a) a nucleic acid encoding a human SNORF33 receptor and having a sequence comprising the sequence of the human SNORF33 nucleic acid contained in plasmid pcDNA3.1-hSNORF33-p (Patent Deposit Designation PTA-101) or (b) a nucleic acid encoding a rat SNORF33 receptor and having a sequence identical to the sequence of the rat SNORF33 receptor-encoding nucleic acid contained in plasmid pcDNA3.1-rSNORF33-f (Patent Deposit Designation PTA-102).

This invention further provides a recombinant nucleic acid comprising a nucleic acid encoding a human SNORF33 receptor, wherein the human SNORF33 receptor comprises an amino acid sequence identical to the sequence encoded by the nucleic acid shown in Figure 1 (SEQ ID NO: 1).

This invention also provides a recombinant nucleic acid comprising a nucleic acid encoding a rat SNORF33 receptor, wherein the rat SNORF33 receptor comprises an amino acid sequence identical to the sequence of the rat SNORF33 receptor encoded by the shortest open reading frame indicated in Figures 3A-3B (SEQ ID NO: 3).

The plasmid pcDNA3.1-hSNORF33-p and plasmid pcDNA3.1-rSNORF33-f were both deposited on May 21, 1999, with the American Type Culture Collection (ATCC), 10801 University Blvd., Manassas, Virginia 20110-2209, U.S.A. under the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure and were accorded Patent Deposit Designation Nos. PTA-101 and PTA-102, respectively.

This invention also provides a recombinant nucleic acid comprising a nucleic acid encoding a mammalian SNORF33 receptor, wherein the mammalian receptor-encoding nucleic acid hybridizes under high stringency conditions to (a) a nucleic acid encoding a human SNORF33 receptor and having a sequence comprising the sequence of the human SNORF33 nucleic acid contained in plasmid pcDNA3.1-hSNORF33-f (Patent Deposit Designation PTA-398) or plasmid pEXJ-hSNORF33-f (Patent Deposit Designation PTA-570).

This invention further provides a recombinant nucleic acid comprising a nucleic acid encoding a human SNORF33 receptor, wherein the human SNORF33 receptor comprises an amino acid sequence identical to the sequence encoded by the nucleic acid shown in Figures 5A-5B (SEQ ID NO: 5).

The plasmid pcDNA3.1-hSNORF33-f was deposited on July 21, 1999, with the American Type Culture Collection (ATCC), 10801 University Blvd., Manassas, Virginia 20110-2209, U.S.A. under the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure and was accorded Patent Deposit Designation PTA-398.

The plasmid pEXJ-hSNORF33-f was deposited on August 24, 1999, with the American Type Culture Collection (ATCC), 10801 University Blvd., Manassas, Virginia 20110-2209, U.S.A. under the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure and was accorded Patent Deposit Designation PTA-570.

Hybridization methods are well known to those of skill in the art. For purposes of this invention, hybridization under high

stringency conditions means hybridization performed at 40°C in a hybridization buffer containing 50% formamide, 5X SSC, 7mM Tris, 1X Denhardt's, 25µg/ml salmon sperm DNA; wash at 50°C in 0.1X SSC, 0.1%SDS.

Throughout this application, the following standard abbreviations are used to indicate specific nucleotide bases:

A = adenine
G = guanine
C = cytosine
T = thymine
M = adenine or cytosine
R = adenine or guanine
W = adenine or thymine
S = cytosine or guanine
Y = cytosine or thymine
K = guanine or thymine
V = adenine, cytosine, or guanine (not thymine)
H = adenine, cytosine, or thymine (not cytosine)
B = cytosine, guanine, or thymine (not adenine)
N = adenine, cytosine, guanine, or thymine (or other modified base such as inosine)
I = inosine

Furthermore, the term "agonist" is used throughout this application to indicate any peptide or non-peptidyl compound which increases the activity of any of the polypeptides of the subject invention. The term "antagonist" is used throughout this application to indicate any peptide or non-peptidyl compound which decreases the activity of any of the polypeptides of the subject invention.

Furthermore, as used herein, the phrase "pharmaceutically acceptable carrier" means any of the standard pharmaceutically

acceptable carriers. Examples include, but are not limited to, phosphate buffered saline, physiological saline, water, and emulsions, such as oil/water emulsions.

It is possible that the mammalian SNORF33 receptor gene contains introns and furthermore, the possibility exists that additional introns could exist in coding or non-coding regions. In addition, spliced form(s) of mRNA may encode additional amino acids either upstream of the currently defined starting methionine or within the coding region. Further, the existence and use of alternative exons is possible, whereby the mRNA may encode different amino acids within the region comprising the exon. In addition, single amino acid substitutions may arise via the mechanism of RNA editing such that the amino acid sequence of the expressed protein is different than that encoded by the original gene. (Burns, et al., 1996; Chu, et al., 1996). Such variants may exhibit pharmacologic properties differing from the polypeptide encoded by the original gene.

This invention provides splice variants of the mammalian SNORF33 receptors disclosed herein. This invention further provides for alternate translation initiation sites and alternately spliced or edited variants of nucleic acids encoding the SNORF33 receptors of this invention.

This invention also contemplates recombinant nucleic acids which comprise nucleic acids encoding naturally occurring allelic variants of the SNORF33 receptors disclosed herein.

The nucleic acids of the subject invention also include nucleic acid analogs of the human SNORF33 receptor genes, wherein the human SNORF33 receptor gene comprises the nucleic acid sequence shown in Figure 1 or contained in plasmid pcDNA3.1-hSNORF33-p (Patent Deposit Designation PTA-101). Nucleic acid analogs of the human SNORF33 receptor genes differ from the human SNORF33

receptor genes described herein in terms of the identity or location of one or more nucleic acid bases (deletion analogs containing less than all of the nucleic acid bases shown in Figure 1 or contained in plasmid pcDNA3.1-hSNORF33-p (Patent Deposit Designation PTA-101), substitution analogs wherein one or more nucleic acid bases shown in Figure 1 or contained in plasmid pcDNA3.1-hSNORF33-p (Patent Deposit Designation PTA-101), are replaced by other nucleic acid bases, and addition analogs, wherein one or more nucleic acid bases are added to a terminal or medial portion of the nucleic acid sequence) and which encode proteins which share some or all of the properties of the proteins encoded by the nucleic acid sequences shown in Figure 1 or contained in plasmid pcDNA3.1-hSNORF33-p (Patent Deposit Designation PTA-101). In one embodiment of the present invention, the nucleic acid analog encodes a protein which has an amino acid sequence identical to that shown in Figure 2 or encoded by the nucleic acid sequence contained in plasmid pcDNA3.1-hSNORF33-p (Patent Deposit Designation PTA-101). In another embodiment, the nucleic acid analog encodes a protein having an amino acid sequence which differs from the amino acid sequences shown in Figure 2 or encoded by the nucleic acid contained in plasmid pcDNA3.1-hSNORF33-p (Patent Deposit Designation PTA-101). In a further embodiment, the protein encoded by the nucleic acid analog has a function which is the same as the function of the receptor proteins having the amino acid sequence shown in Figure 2. In another embodiment, the function of the protein encoded by the nucleic acid analog differs from the function of the receptor protein having the amino acid sequence shown in Figure 2. In another embodiment, the variation in the nucleic acid sequence occurs within the transmembrane (TM) region of the protein. In a further embodiment, the variation in the nucleic acid sequence occurs outside of the TM region.

The nucleic acids of the subject invention also include nucleic

acid analogs of the rat SNORF33 receptor genes, wherein the rat SNORF33 receptor gene comprises the nucleic acid sequence shown in Figures 3A-3B or contained in plasmid pcDNA3.1-rSNORF33-f (Patent Deposit Designation PTA-102). Nucleic acid analogs of the rat SNORF33 receptor genes differ from the rat SNORF33 receptor genes described herein in terms of the identity or location of one or more nucleic acid bases (deletion analogs containing less than all of the nucleic acid bases shown in Figures 3A-3B or contained in plasmid pcDNA3.1-rSNORF33-f (Patent Deposit Designation PTA-102) substitution analogs wherein one or more nucleic acid bases shown in Figures 3A-3B or contained in plasmid pcDNA3.1-rSNORF33-f (Patent Deposit Designation PTA-102), are replaced by other nucleic acid bases, and addition analogs, wherein one or more nucleic acid bases are added to a terminal or medial portion of the nucleic acid sequence) and which encode proteins which share some or all of the properties of the proteins encoded by the nucleic acid sequences shown in Figure 3A-3B or contained in plasmid pcDNA3.1-rSNORF33-f (Patent Deposit Designation PTA-102). In one embodiment of the present invention, the nucleic acid analog encodes a protein which has an amino acid sequence identical to that shown in Figures 4A-4B or encoded by the nucleic acid sequence contained in plasmid pcDNA3.1-rSNORF33-f (Patent Deposit Designation PTA-102). In another embodiment, the nucleic acid analog encodes a protein having an amino acid sequence which differs from the amino acid sequences shown in Figures 4A-4B or encoded by the nucleic acid contained in plasmid pcDNA3.1-rSNORF33-f (Patent Deposit Designation PTA-102). In a further embodiment, the protein encoded by the nucleic acid analog has a function which is the same as the function of the receptor proteins having the amino acid sequence shown in Figures 4A-4B. In another embodiment, the function of the protein encoded by the nucleic acid analog differs from the function of the receptor protein having the amino acid sequence shown in Figures 4A-4B. In another embodiment, the variation in the nucleic acid sequence occurs within the transmembrane (TM) region

of the protein. In a further embodiment, the variation in the nucleic acid sequence occurs outside of the TM region.

The nucleic acids of the subject invention also include nucleic acid analogs of the human SNORF33 receptor genes, wherein the human SNORF33 receptor gene comprises the nucleic acid sequence shown in Figures 5A-5B or contained in plasmid pcDNA3.1-hSNORF33-f (Patent Deposit Designation PTA-398) or plasmid pEXJ-hSNORF33-f (Patent Deposit Designation PTA-570). Nucleic acid analogs of the human SNORF33 receptor genes differ from the human SNORF33 receptor genes described herein in terms of the identity or location of one or more nucleic acid bases (deletion analogs containing less than all of the nucleic acid bases shown in Figures 5A-5B or contained in plasmid pcDNA3.1-hSNORF33-f (Patent Deposit Designation PTA-398) or plasmid pEXJ-hSNORF33-f (Patent Deposit Designation PTA-570), substitution analogs wherein one or more nucleic acid bases shown in Figures 5A-5B or contained in plasmid pcDNA3.1-hSNORF33-f (Patent Deposit Designation PTA-398) or plasmid pEXJ-hSNORF33-f (Patent Deposit Designation PTA-570), are replaced by other nucleic acid bases, and addition analogs, wherein one or more nucleic acid bases are added to a terminal or medial portion of the nucleic acid sequence) and which encode proteins which share some or all of the properties of the proteins encoded by the nucleic acid sequences shown in Figures 5A-5B or contained in plasmid pcDNA3.1-hSNORF33-f (Patent Deposit Designation PTA-398) or plasmid pEXJ-hSNORF33-f (Patent Deposit Designation PTA-570). In one embodiment of the present invention, the nucleic acid analog encodes a protein which has an amino acid sequence identical to that shown in Figures 6A-6B or encoded by the nucleic acid sequence contained in plasmid pcDNA3.1-hSNORF33-f (Patent Deposit Designation PTA-398) or plasmid pEXJ-hSNORF33-f (Patent Deposit Designation PTA-570). In another embodiment, the nucleic acid analog encodes a protein having an amino acid sequence which differs from the amino acid sequences shown in Figures 6A-6B or encoded by the nucleic acid

contained in plasmid pcDNA3.1-hSNORF33-f (Patent Deposit Designation PTA-398) or plasmid pEXJ-hSNORF33-f (Patent Deposit Designation PTA-570). In a further embodiment, the protein encoded by the nucleic acid analog has a function which is the same as the function of the receptor proteins having the amino acid sequence shown in Figures 6A-6B. In another embodiment, the function of the protein encoded by the nucleic acid analog differs from the function of the receptor protein having the amino acid sequence shown in Figures 6A-6B. In another embodiment, the variation in the nucleic acid sequence occurs within the transmembrane (TM) region of the protein. In a further embodiment, the variation in the nucleic acid sequence occurs outside of the TM region.

This invention provides the above-described isolated nucleic acid, wherein the nucleic acid is DNA. In an embodiment, the DNA is cDNA. In another embodiment, the DNA is genomic DNA. In still another embodiment, the nucleic acid is RNA. Methods for production and manipulation of nucleic acid molecules are well known in the art.

This invention further provides nucleic acid which is degenerate with respect to the DNA encoding any of the polypeptides described herein. In an embodiment, the nucleic acid comprises a nucleotide sequence which is degenerate with respect to the nucleotide sequence shown in Figure 1 (SEQ ID NO: 1) or the nucleotide sequence contained in the plasmid pcDNA3.1-hSNORF33-p (Patent Deposit Designation PTA-101), that is, a nucleotide sequence which is translated into the same amino acid sequence. In another embodiment, the nucleic acid comprises a nucleotide sequence which is degenerate with respect to the nucleotide sequence shown in Figures 5A-5B (SEQ ID NO: 5) or the nucleotide sequence contained in the plasmid pcDNA3.1-hSNORF33-f (Patent Deposit Designation PTA-398) or the plasmid pEXJ-hSNORF33-f (Patent Deposit Designation PTA-570), that is, a nucleotide

sequence which is translated into the same amino acid sequence.

This invention further provides nucleic acid which is degenerate with respect to the DNA encoding any of the polypeptides described herein. In an embodiment, the nucleic acid comprises a nucleotide sequence which is degenerate with respect to the nucleotide sequence shown in Figures 3A-3B (SEQ ID NO: 3) or the nucleotide sequence contained in the plasmid pcDNA3.1-rSNORF33-f (Patent Deposit Designation PTA-102), that is, a nucleotide sequence which is translated into the same amino acid sequence.

This invention also encompasses DNAs and cDNAs which encode amino acid sequences which differ from those of the polypeptides of this invention, but which should not produce phenotypic changes.

Alternately, this invention also encompasses DNAs, cDNAs, and RNAs which hybridize to the DNA, cDNA, and RNA of the subject invention. Hybridization methods are well known to those of skill in the art.

The nucleic acids of the subject invention also include nucleic acid molecules coding for polypeptide analogs, fragments or derivatives of antigenic polypeptides which differ from naturally-occurring forms in terms of the identity or location of one or more amino acid residues (deletion analogs containing less than all of the residues specified for the protein, substitution analogs wherein one or more residues specified are replaced by other residues and addition analogs wherein one or more amino acid residues is added to a terminal or medial portion of the polypeptides) and which share some or all properties of naturally-occurring forms. These molecules include: the incorporation of codons "preferred" for expression by selected non-mammalian hosts; the provision of sites for cleavage by restriction endonuclease enzymes; and the provision of additional initial, terminal or intermediate DNA sequences that facilitate

construction of readily expressed vectors. The creation of polypeptide analogs is well known to those of skill in the art (Spurney, R. F. et al. (1997); Fong, T.M. et al. (1995); Underwood, D.J. et al. (1994); Graziano, M.P. et al. (1996); Guan X.M. et al. (1995)).

The modified polypeptides of this invention may be transfected into cells either transiently or stably using methods well-known in the art, examples of which are disclosed herein. This invention also provides for binding assays using the modified polypeptides, in which the polypeptide is expressed either transiently or in stable cell lines. This invention further provides a compound identified using a modified polypeptide in a binding assay such as the binding assays described herein.

The nucleic acids described and claimed herein are useful for the information which they provide concerning the amino acid sequence of the polypeptide and as products for the large scale synthesis of the polypeptides by a variety of recombinant techniques. The nucleic acid molecule is useful for generating new cloning and expression vectors, transformed and transfected prokaryotic and eukaryotic host cells, and new and useful methods for cultured growth of such host cells capable of expression of the polypeptide and related products.

This invention also provides an isolated nucleic acid encoding species homologs of the SNORF33 receptor encoded by the nucleic acid sequence shown in Figure 1 (SEQ ID NO: 1) or encoded by the plasmid pcDNA3.1-hSNORF33-p (Patent Deposit Designation PTA-101). In one embodiment, the nucleic acid encodes a mammalian SNORF33 receptor homolog which has substantially the same amino acid sequence as does the SNORF33 receptor encoded by the plasmid pcDNA3.1-hSNORF33-p (Patent Deposit Designation PTA-101). In another embodiment, the nucleic acid encodes a mammalian SNORF33 receptor homolog which has above 75% amino acid identity to the

SNORF33 receptor encoded by the plasmid pcDNA3.1-hSNORF33-p (Patent Deposit Designation PTA-101); preferably above 85% amino acid identity to the SNORF33 receptor encoded by the plasmid pcDNA3.1-hSNORF33-p (Patent Deposit Designation PTA-101); most preferably above 95% amino acid identity to the SNORF33 receptor encoded by the plasmid pcDNA3.1-hSNORF33-p (Patent Deposit Designation PTA-101). In another embodiment, the mammalian SNORF33 receptor homolog has above 70% nucleic acid identity to the SNORF33 receptor gene contained in plasmid pcDNA3.1-hSNORF33-p (Patent Deposit Designation PTA-101); preferably above 80% nucleic acid identity to the SNORF33 receptor gene contained in the plasmid pcDNA3.1-hSNORF33-p (Patent Deposit Designation PTA-101); more preferably above 90% nucleic acid identity to the SNORF33 receptor gene contained in the plasmid pcDNA3.1-hSNORF33-p (Patent Deposit Designation PTA-101). Examples of methods for isolating and purifying species homologs are described elsewhere (e.g., U.S. Patent No. 5,602,024, WO94/14957, WO97/26853, WO98/15570).

This invention also provides an isolated nucleic acid encoding species homologs of the SNORF33 receptors encoded by the nucleic acid sequence shown in Figures 5A-5B (SEQ ID NO: 5) or encoded by the plasmid pcDNA3.1-hSNORF33-f (Patent Deposit Designation PTA-398). In one embodiment, the nucleic acid encodes a mammalian SNORF33 receptor homolog which has substantially the same amino acid sequence as does the SNORF33 receptor encoded by the plasmid pcDNA3.1-hSNORF33-f (Patent Deposit Designation PTA-398). In another embodiment, the nucleic acid encodes a mammalian SNORF33 receptor homolog which has above 75% amino acid identity to the SNORF33 receptor encoded by the pcDNA3.1-hSNORF33-f (Patent Deposit Designation PTA-398); preferably above 85% amino acid identity to the SNORF33 receptor encoded by the plasmid pcDNA3.1-hSNORF33-f (Patent Deposit Designation PTA-398; most preferably above 95% amino acid identity to the SNORF33 receptor encoded by the plasmid pcDNA3.1-hSNORF33-f (Patent Deposit Designation PTA-

398). In another embodiment, the mammalian SNORF33 receptor homolog has above 70% nucleic acid identity to the SNORF33 receptor gene contained in plasmid pcDNA3.1-hSNORF33-f (Patent Deposit Designation PTA-398); preferably above 80% nucleic acid identity to the SNORF33 receptor gene contained in the plasmid pcDNA3.1-hSNORF33-f (Patent Deposit Designation PTA-398); more preferably above 90% nucleic acid identity to the SNORF33 receptor gene contained in the plasmid pcDNA3.1-hSNORF33-f (Patent Deposit Designation PTA-398).

This invention also provides an isolated nucleic acid encoding species homologs of the SNORF33 receptors encoded by the nucleic acid sequence shown in Figures 5A-5B (SEQ ID NO: 5) or encoded by the plasmid pEXJ-hSNORF33-f (Patent Deposit Designation PTA-570). In one embodiment, the nucleic acid encodes a mammalian SNORF33 receptor homolog which has substantially the same amino acid sequence as does the SNORF33 receptor encoded by the plasmid pEXJ-hSNORF33-f (Patent Deposit Designation PTA-570). In another embodiment, the nucleic acid encodes a mammalian SNORF33 receptor homolog which has above 75% amino acid identity to the SNORF33 receptor encoded by the plasmid pEXJ-hSNORF33-f (Patent Deposit Designation PTA-570); preferably above 85% amino acid identity to the SNORF33 receptor encoded by the plasmid pEXJ-hSNORF33-f (Patent Deposit Designation PTA-570); most preferably above 95% amino acid identity to the SNORF33 receptor encoded by the plasmid pEXJ-hSNORF33-f (Patent Deposit Designation PTA-570). In another embodiment, the mammalian SNORF33 receptor homolog has above 70% nucleic acid identity to the SNORF33 receptor gene contained in plasmid pEXJ-hSNORF33-f (Patent Deposit Designation PTA-570); preferably above 80% nucleic acid identity to the SNORF33 receptor gene contained in the plasmid pEXJ-hSNORF33-f (Patent Deposit Designation PTA-570); more preferably above 90% nucleic acid identity to the SNORF33 receptor gene contained in the plasmid pEXJ-hSNORF33-f (Patent Deposit Designation PTA-570).

This invention also provides an isolated nucleic acid encoding species homologs of the SNORF33 receptors encoded by the nucleic acid sequence shown in Figures 3A-3B (SEQ ID NO: 3) or encoded by the plasmid pcDNA3.1-rSNORF33-f (Patent Deposit Designation PTA-102). In one embodiment, the nucleic acid encodes a mammalian SNORF33 receptor homolog which has substantially the same amino acid sequence as does the SNORF33 receptor encoded by the plasmid pcDNA3.1-rSNORF33-f (Patent Deposit Designation PTA-102). In another embodiment, the nucleic acid encodes a mammalian SNORF33 receptor homolog which has above 75% amino acid identity to the SNORF33 receptor encoded by the pcDNA3.1-rSNORF33-f (Patent Deposit Designation PTA-102); preferably above 85% amino acid identity to the SNORF33 receptor encoded by the plasmid pcDNA3.1-rSNORF33-f (Patent Deposit Designation PTA-102; most preferably above 95% amino acid identity to the SNORF33 receptor encoded by the plasmid pcDNA3.1-rSNORF33-f (Patent Deposit Designation PTA-102). In another embodiment, the mammalian SNORF33 receptor homolog has above 70% nucleic acid identity to the SNORF33 receptor gene contained in plasmid pcDNA3.1-rSNORF33-f (Patent Deposit Designation PTA-102); preferably above 80% nucleic acid identity to the SNORF33 receptor gene contained in the plasmid pcDNA3.1-rSNORF33-f (Patent Deposit Designation PTA-102); more preferably above 90% nucleic acid identity to the SNORF33 receptor gene contained in the plasmid pcDNA3.1-rSNORF33-f (Patent Deposit Designation PTA-102).

This invention provides an isolated nucleic acid encoding a modified mammalian SNORF33 receptor, which differs from a mammalian SNORF33 receptor by having an amino acid(s) deletion, replacement, or addition in the third intracellular domain.

This invention provides an isolated nucleic acid encoding a mammalian SNORF33 receptor. In one embodiment, the nucleic acid is DNA. In another embodiment, the DNA is cDNA. In another embodiment, the DNA is genomic DNA. In another embodiment, the

nucleic acid is RNA. In another embodiment, the mammalian SNORF33 receptor is a human SNORF33 receptor. In another embodiment, the human SNORF33 receptor has an amino acid sequence identical to that encoded by the plasmid pcDNA3.1-hSNORF33-p (Patent Deposit Designation PTA-101), the plasmid pcDNA3.1-hSNORF33-f (Patent Deposit Designation PTA-398) or the plasmid pEXJ-hSNORF33-f (Patent Deposit Designation PTA-570). In another embodiment, the human SNORF33 receptor has an amino acid sequence identical to the amino acid sequence shown in Figure 2 (SEQ ID NO: 2) or Figures 6A-6B (SEQ ID NO: 6).

In an embodiment, the mammalian SNORF33 receptor is a rat SNORF33 receptor. In another embodiment, the rat SNORF33 receptor has an amino acid sequence identical to that encoded by the plasmid pcDNA3.1-rSNORF33-f (Patent Deposit Designation PTA-102). In another embodiment, the rat SNORF33 receptor has an amino acid sequence identical to the amino acid sequence shown in Figures 4A-4B (SEQ ID NO: 4).

This invention provides a purified mammalian SNORF33 receptor protein. In one embodiment, the SNORF33 receptor protein is a human SNORF33 receptor protein. In a further embodiment, the SNORF33 receptor protein is a rat SNORF33 receptor protein.

This invention provides a vector comprising the nucleic acid of this invention. This invention further provides a vector adapted for expression in a cell which comprises the regulatory elements necessary for expression of the nucleic acid in the cell operatively linked to the nucleic acid encoding the receptor so as to permit expression thereof, wherein the cell is a bacterial, amphibian, yeast, insect or mammalian cell. In one embodiment, the vector is a baculovirus. In another embodiment, the vector is a plasmid.

This invention provides a plasmid designated pcDNA3.1-hSNORF33-p

(Patent Deposit Designation PTA-101). This invention also provides a plasmid designated pcDNA3.1-rSNORF33-f (Patent Deposit Designation PTA-102). This invention provides a plasmid designated pcDNA3.1-hSNORF33-f (Patent Deposit Designation PTA-398). This invention provides a plasmid pEXJ-hSNORF33-f (Patent Deposit Designation PTA-570).

This invention further provides for any vector or plasmid which comprises modified untranslated sequences, which are beneficial for expression in desired host cells or for use in binding or functional assays. For example, a vector or plasmid with untranslated sequences of varying lengths may express differing amounts of the polypeptide depending upon the host cell used. In an embodiment, the vector or plasmid comprises the coding sequence of the polypeptide and the regulatory elements necessary for expression in the host cell.

This invention provides for a cell comprising the vector of this invention. In one embodiment, the cell is a non-mammalian cell. In one embodiment, the non-mammalian cell is a *Xenopus* oocyte cell or a *Xenopus* melanophore cell. In another embodiment, the cell is a mammalian cell. In another embodiment, the cell is a COS-7 cell, a 293 human embryonic kidney cell, a NIH-3T3 cell, a LM(tk-) cell, a mouse Y1 cell, or a CHO cell. In another embodiment, the cell is an insect cell. In another embodiment, the insect cell is an Sf9 cell, an Sf21 cell or a Trichoplusia ni 5B-4 cell.

This invention provides a membrane preparation isolated from the cell in accordance with this invention.

Furthermore, this invention provides for a nucleic acid probe comprising at least 15 nucleotides, which probe specifically hybridizes with a nucleic acid encoding a mammalian SNORF33 receptor, wherein the probe has a sequence complementary to a

unique sequence present within one of the two strands of the nucleic acid encoding the mammalian SNORF33 receptor contained in plasmid pcDNA3.1-hSNORF33-p (Patent Deposit Designation PTA-101), plasmid pcDNA3.1-rSNORF33-f (Patent Deposit Designation PTA-102), plasmid pcDNA3.1-hSNORF33-f (Patent Deposit Designation PTA-398) or plasmid pEXJ-hSNORF33-f (Patent Deposit Designation PTA-570).

This invention further provides a nucleic acid probe comprising at least 15 nucleotides, which probe specifically hybridizes with a nucleic acid encoding a mammalian SNORF33 receptor, wherein the probe has a sequence complementary to a unique sequence present within (a) the nucleic acid sequence shown in Figure 1 (SEQ ID NO: 1) or (b) the reverse complement thereof. This invention further provides a nucleic acid probe comprising at least 15 nucleotides, which probe specifically hybridizes with a nucleic acid encoding a mammalian SNORF33 receptor, wherein the probe has a sequence complementary to a unique sequence present within (a) the nucleic acid sequence shown in Figures 5A-5B (SEQ ID NO: 5) or (b) the reverse complement thereof. This invention also provides a nucleic acid probe comprising at least 15 nucleotides, which probe specifically hybridizes with a nucleic acid encoding a mammalian SNORF33 receptor, wherein the probe has a sequence complementary to a unique sequence present within (a) the nucleic acid sequence shown in Figures 3A-3B (SEQ ID NO: 3) or (b) the reverse complement thereof. In one embodiment, the nucleic acid is DNA. In another embodiment, the nucleic acid is RNA.

As used herein, the phrase "specifically hybridizing" means the ability of a nucleic acid molecule to recognize a nucleic acid sequence complementary to its own and to form double-helical segments through hydrogen bonding between complementary base pairs.

The nucleic acids of this invention may be used as probes to

obtain homologous nucleic acids from other species and to detect the existence of nucleic acids having complementary sequences in samples.

The nucleic acids may also be used to express the receptors they encode in transfected cells.

The use of a constitutively active receptor encoded by SNORF33 either occurring naturally without further modification or after appropriate point mutations, deletions or the like, allows screening for antagonists and *in vivo* use of such antagonists to attribute a role to receptor SNORF33 without prior knowledge of the endogenous ligand.

Use of the nucleic acids further enables elucidation of possible receptor diversity and of the existence of multiple subtypes within a family of receptors of which SNORF33 is a member.

Finally, it is contemplated that this receptor will serve as a valuable tool for designing drugs for treating various pathophysiological conditions such as chronic and acute inflammation, arthritis, autoimmune diseases, transplant rejection, graft vs. host disease, bacterial, fungal, protozoan and viral infections, septicemia, AIDS, pain, psychotic and neurological disorders, including anxiety, depression, schizophrenia, dementia, mental retardation, memory loss, epilepsy, neuromotor disorders, respiratory disorders, asthma, eating/body weight disorders including obesity, bulimia, diabetes, anorexia, nausea, hypertension, hypotension, vascular and cardiovascular disorders, ischemia, stroke, cancers, ulcers, urinary retention, sexual/reproductive disorders, circadian rhythm disorders, renal disorders, bone diseases including osteoporosis, benign prostatic hypertrophy, gastrointestinal disorders, nasal congestion, dermatological disorders such as psoriasis, allergies, Parkinson's disease, Alzheimer's disease,

acute heart failure, angina disorders, delirium, dyskinesias such as Huntington's disease or Gille's de la Tourette's syndrome, among others and diagnostic assays for such conditions. This receptor may also serve as a valuable tool for designing drugs for chemoprevention.

Methods of transfecting cells e.g. mammalian cells, with such nucleic acid to obtain cells in which the receptor is expressed on the surface of the cell are well known in the art. (See, for example, U.S. Patent Nos. 5,053,337; 5,155,218; 5,360,735; 5,472,866; 5,476,782; 5,516,653; 5,545,549; 5,556,753; 5,595,880; 5,602,024; 5,639,652; 5,652,113; 5,661,024; 5,766,879; 5,786,155; and 5,786,157, the disclosures of which are hereby incorporated by reference in their entireties into this application.)

Such transfected cells may also be used to test compounds and screen compound libraries to obtain compounds which bind to the SNORF33 receptor, as well as compounds which activate or inhibit activation of functional responses in such cells, and therefore are likely to do so *in vivo*. (See, for example, U.S. Patent Nos. 5,053,337; 5,155,218; 5,360,735; 5,472,866; 5,476,782; 5,516,653; 5,545,549; 5,556,753; 5,595,880; 5,602,024; 5,639,652; 5,652,113; 5,661,024; 5,766,879; 5,786,155; and 5,786,157, the disclosures of which are hereby incorporated by reference in their entireties into this application.)

This invention further provides an antibody capable of binding to a mammalian receptor encoded by a nucleic acid encoding a mammalian receptor. In one embodiment, the mammalian receptor is a human receptor. In a further embodiment , the mammalian receptor is a rat receptor. This invention also provides an agent capable of competitively inhibiting the binding of the antibody to a mammalian receptor. In one embodiment, the antibody is a monoclonal antibody or antisera.

Methods of preparing and employing antisense oligonucleotides, antibodies, nucleic acid probes and transgenic animals directed to the SNORF33 receptor are well known in the art. (See, for example, U.S. Patent Nos. 5,053,337; 5,155,218; 5,360,735; 5,472,866; 5,476,782; 5,516,653; 5,545,549; 5,556,753; 5,595,880; 5,602,024; 5,639,652; 5,652,113; 5,661,024; 5,766,879; 5,786,155; and 5,786,157, the disclosures of which are hereby incorporated by reference in their entireties into this application.)

This invention provides for an antisense oligonucleotide having a sequence capable of specifically hybridizing to RNA encoding a mammalian SNORF33 receptor, so as to prevent translation of such RNA. This invention further provides for an antisense oligonucleotide having a sequence capable of specifically hybridizing to genomic DNA encoding a mammalian SNORF33 receptor, so as to prevent transcription of such genomic DNA. In one embodiment, the oligonucleotide comprises chemically modified nucleotides or nucleotide analogues.

This invention also provides for an antibody capable of binding to a mammalian SNORF33 receptor encoded by a nucleic acid in accordance with this invention. In one embodiment, the mammalian SNORF33 receptor is a human SNORF33 receptor. In a further embodiment, the mammalian SNORF33 receptor is a rat SNORF33 receptor.

Moreover, this invention provides an agent capable of competitively inhibiting the binding of an antibody in accordance with this invention to a mammalian SNORF33 receptor. In one embodiment, the antibody is a monoclonal antibody or antisera.

This invention still further provides a pharmaceutical composition comprising (a) an amount of an oligonucleotide in accordance with this invention capable of passing through a cell membrane and effective to reduce expression of a mammalian

SNORF33 receptor and (b) a pharmaceutically acceptable carrier capable of passing through the cell membrane.

In one embodiment, the oligonucleotide is coupled to a substance which inactivates mRNA. In another embodiment, the substance which inactivates mRNA is a ribozyme. In another embodiment, the pharmaceutically acceptable carrier comprises a structure which binds to a mammalian SNORF33 receptor on a cell capable of being taken up by the cells after binding to the structure. In another embodiment, the pharmaceutically acceptable carrier is capable of binding to a mammalian SNORF33 receptor which is specific for a selected cell type.

This invention also provides a pharmaceutical composition which comprises an amount of an antibody in accordance with this invention effective to block binding of a ligand to a human SNORF33 receptor and a pharmaceutically acceptable carrier.

This invention further provides a transgenic, nonhuman mammal expressing DNA encoding a mammalian SNORF33 receptor in accordance with this invention. This invention provides a transgenic, nonhuman mammal comprising a homologous recombination knockout of a native mammalian SNORF33 receptor. This invention further provides a transgenic, nonhuman mammal whose genome comprises antisense DNA complementary to DNA encoding a mammalian SNORF33 receptor in accordance with this invention so placed within such genome as to be transcribed into antisense mRNA which is complementary and hybridizes with mRNA encoding the mammalian SNORF33 receptor so as to thereby reduce translation or such mRNA and expression of such receptor. In one embodiment, the DNA encoding the mammalian SNORF33 receptor additionally comprises an inducible promoter. In another embodiment, the DNA encoding the mammalian SNORF33 receptor additionally comprises tissue specific regulatory elements. In another embodiment, the transgenic, nonhuman mammal is a mouse.

This invention provides for a process for identifying a chemical compound which specifically binds to a mammalian SNORF33 receptor which comprises contacting cells containing DNA encoding, and expressing on their cell surface, the mammalian SNORF33 receptor, wherein such cells do not normally express the mammalian SNORF33 receptor, with the compound under conditions suitable for binding, and detecting specific binding of the chemical compound to the mammalian SNORF33 receptor. This invention further provides for a process for identifying a chemical compound which specifically binds to a mammalian SNORF33 receptor which comprises contacting a membrane preparation from cells containing DNA encoding and expressing on their cell surface the mammalian SNORF33 receptor, wherein such cells do not normally express the mammalian SNORF33 receptor, with the compound under conditions suitable for binding, and detecting specific binding of the chemical compound to the mammalian SNORF33 receptor.

In one embodiment, the mammalian SNORF33 receptor is a human SNORF33 receptor. In another embodiment, the mammalian SNORF33 receptor has substantially the same amino acid sequence as the human SNORF33 receptor encoded by plasmid pcDNA3.1-hSNORF33-f (Patent Deposit Designation PTA-398). In another embodiment, the mammalian SNORF33 receptor has substantially the same amino acid sequence as the human SNORF33 receptor encoded by plasmid pEXJ-hSNORF33-f (Patent Deposit Designation PTA-570). In another embodiment, the mammalian SNORF33 receptor has substantially the same amino acid sequence as that shown in Figures 6A-6B (SEQ ID NO: 6). In another embodiment, the mammalian SNORF33 receptor has the amino acid sequence shown in Figures 6A-6B (SEQ ID NO: 6).

In another embodiment, the mammalian SNORF33 receptor is a rat SNORF33 receptor. In another embodiment, the mammalian SNORF33 receptor has substantially the same amino acid sequence as the

rat SNORF33 receptor encoded by plasmid pcDNA3.1-rSNORF33-f (Patent Deposit Designation PTA-102). In another embodiment, the mammalian SNORF33 receptor has substantially the same amino acid sequence as that shown in Figures 4A-4B (SEQ ID NO: 4). In another embodiment, the mammalian SNORF33 receptor has the amino acid sequence shown in Figures 4A-4B (SEQ ID NO: 4).

In one embodiment, the compound is not previously known to bind to a mammalian SNORF33 receptor. In one embodiment, the cell is an insect cell. In one embodiment, the cell is a mammalian cell. In another embodiment, the cell is nonneuronal in origin. In another embodiment, the nonneuronal cell is a COS-7 cell, 293 human embryonic kidney cell, a CHO cell, a NIH-3T3 cell, a mouse Y1 cell, or a LM(tk-) cell. In another embodiment, the compound is a compound not previously known to bind to a mammalian SNORF33 receptor. This invention provides a compound identified by the preceding process according to this invention.

This invention still further provides a process involving competitive binding for identifying a chemical compound which specifically binds to a mammalian SNORF33 receptor which comprises separately contacting cells expressing on their cell surface the mammalian SNORF33 receptor, wherein such cells do not normally express the mammalian SNORF33 receptor, with both the chemical compound and a second chemical compound known to bind to the receptor, and with only the second chemical compound, under conditions suitable for binding of such compounds to the receptor, and detecting specific binding of the chemical compound to the mammalian SNORF33 receptor, a decrease in the binding of the second chemical compound to the mammalian SNORF33 receptor in the presence of the chemical compound being tested indicating that such chemical compound binds to the mammalian SNORF33 receptor.

This invention provides a process involving competitive binding

for identifying a chemical compound which specifically binds to a mammalian SNORF33 receptor which comprises separately contacting a membrane preparation from cells expressing on their cell surface the mammalian SNORF33 receptor, wherein such cells do not normally express the mammalian SNORF33 receptor, with both the chemical compound and a second chemical compound known to bind to the receptor, and with only the second chemical compound, under conditions suitable for binding of such compounds to the receptor, and detecting specific binding of the chemical compound to the mammalian SNORF33 receptor, a decrease in the binding of the second chemical compound to the mammalian SNORF33 receptor in the presence of the chemical compound being tested indicating that such chemical compound binds to the mammalian SNORF33 receptor.

In an embodiment of the present invention, the second chemical compound is a trace amine. Examples of trace amines include, but are not limited to, tryptamine (TYR), tyramine (T), octopamine (OCT), and β-phenyl-ethylamine (PEA).

In one embodiment, the mammalian SNORF33 receptor is a human SNORF33 receptor. In another embodiment, the mammalian SNORF33 receptor is a rat SNORF33 receptor. In a further embodiment, the cell is an insect cell. In another embodiment, the cell is a mammalian cell. In another embodiment, the cell is nonneuronal in origin. In another embodiment, the nonneuronal cell is a COS-7 cell, 293 human embryonic kidney cell, a CHO cell, a NIH-3T3 cell, a mouse Y1 cell, or a LM(tk-) cell. In another embodiment, the compound is not previously known to bind to a mammalian SNORF33 receptor. This invention provides for a compound identified by the preceding process according to this invention.

This invention provides for a method of screening a plurality of chemical compounds not known to bind to a mammalian SNORF33

receptor to identify a compound which specifically binds to the mammalian SNORF33 receptor, which comprises (a) contacting cells transfected with, and expressing, DNA encoding the mammalian SNORF33 receptor with a compound known to bind specifically to the mammalian SNORF33 receptor; (b) contacting the cells of step (a) with the plurality of compounds not known to bind specifically to the mammalian SNORF33 receptor, under conditions permitting binding of compounds known to bind to the mammalian SNORF33 receptor; (c) determining whether the binding of the compound known to bind to the mammalian SNORF33 receptor is reduced in the presence of the plurality of compounds, relative to the binding of the compound in the absence of the plurality of compounds; and if so (d) separately determining the binding to the mammalian SNORF33 receptor of each compound included in the plurality of compounds, so as to thereby identify any compound included therein which specifically binds to the mammalian SNORF33 receptor.

This invention provides a method of screening a plurality of chemical compounds not known to bind to a mammalian SNORF33 receptor to identify a compound which specifically binds to the mammalian SNORF33 receptor, which comprises (a) contacting a membrane preparation from cells transfected with, and expressing, DNA encoding the mammalian SNORF33 receptor with the plurality of compounds not known to bind specifically to the mammalian SNORF33 receptor under conditions permitting binding of compounds known to bind to the mammalian SNORF33 receptor; (b) determining whether the binding of a compound known to bind to the mammalian SNORF33 receptor is reduced in the presence of the plurality of compounds, relative to the binding of the compound in the absence of the plurality of compounds; and if so (c) separately determining the binding to the mammalian SNORF33 receptor of each compound included in the plurality of compounds, so as to thereby identify any compound included therein which specifically binds to the mammalian SNORF33 receptor.

In one embodiment, the mammalian SNORF33 receptor is a human SNORF33 receptor. In a further embodiment, the mammalian SNORF33 receptor is a rat SNORF33 receptor. In another embodiment, the cell is a mammalian cell. In another embodiment, the mammalian cell is non-neuronal in origin. In a further embodiment, the non-neuronal cell is a COS-7 cell, a 293 human embryonic kidney cell, a LM(tk-) cell, a CHO cell, a mouse Y1 cell, or an NIH-3T3 cell.

This invention also provides a method of detecting expression of a mammalian SNORF33 receptor by detecting the presence of mRNA coding for the mammalian SNORF33 receptor which comprises obtaining total mRNA from the cell and contacting the mRNA so obtained with a nucleic acid probe according to this invention under hybridizing conditions, detecting the presence of mRNA hybridized to the probe, and thereby detecting the expression of the mammalian SNORF33 receptor by the cell.

This invention further provides for a method of detecting the presence of a mammalian SNORF33 receptor on the surface of a cell which comprises contacting the cell with an antibody according to this invention under conditions permitting binding of the antibody to the receptor, detecting the presence of the antibody bound to the cell, and thereby detecting the presence of the mammalian SNORF33 receptor on the surface of the cell.

This invention still further provides a method of determining the physiological effects of varying levels of activity of mammalian SNORF33 receptors which comprises producing a transgenic, nonhuman mammal in accordance with this invention whose levels of mammalian SNORF33 receptor activity are varied by use of an inducible promoter which regulates mammalian SNORF33 receptor expression.

This invention additionally provides a method of determining the physiological effects of varying levels of activity of mammalian SNORF33 receptors which comprises producing a panel of transgenic, nonhuman mammals in accordance with this invention each expressing a different amount of mammalian SNORF33 receptor.

Moreover, this invention provides method for identifying an antagonist capable of alleviating an abnormality wherein the abnormality is alleviated by decreasing the activity of a mammalian SNORF33 receptor comprising administering a compound to a transgenic, nonhuman mammal according to this invention, and determining whether the compound alleviates any physiological and/or behavioral abnormality displayed by the transgenic, nonhuman mammal as a result of overactivity of a mammalian SNORF33 receptor, the alleviation of such an abnormality identifying the compound as an antagonist. In one embodiment, the mammalian SNORF33 receptor is a human SNORF33 receptor. In a further embodiment, the mammalian SNORF33 receptor is a rat SNORF33 receptor. The invention also provides an antagonist identified by the preceding method according to this invention. This invention further provides a composition, e.g. a pharmaceutical composition comprising an antagonist according to this invention and a carrier, e.g. a pharmaceutically acceptable carrier. This invention provides a method of treating an abnormality in a subject wherein the abnormality is alleviated by decreasing the activity of a mammalian SNORF33 receptor which comprises administering to the subject an effective amount of the pharmaceutical composition according to this invention so as to thereby treat the abnormality.

In addition, this invention provides a method for identifying an agonist capable of alleviating an abnormality in a subject wherein the abnormality is alleviated by increasing the activity of a mammalian SNORF33 receptor comprising administering a compound to a transgenic, nonhuman mammal according to this

invention, and determining whether the compound alleviates any physiological and/or behavioral abnormality displayed by the transgenic, nonhuman mammal, the alleviation of such an abnormality identifying the compound as an agonist. In one embodiment, the mammalian SNORF33 receptor is a human SNORF33 receptor. In a further embodiment, the mammalian SNORF33 receptor is a rat SNORF33 receptor. This invention provides an agonist identified by the preceding method according to this invention. This invention provides a composition, e.g. a pharmaceutical composition comprising an agonist identified by a method according to this invention and a carrier, e.g. a pharmaceutically acceptable carrier..

Moreover, this invention provides a method of treating an abnormality in a subject wherein the abnormality is alleviated by increasing the activity of a mammalian SNORF33 receptor which comprises administering to the subject an effective amount of the pharmaceutical composition of this invention so as to thereby treat the abnormality.

Yet further, this invention provides a method for diagnosing a predisposition to a disorder associated with the activity of a specific mammalian allele which comprises: (a) obtaining DNA of subjects suffering from the disorder; (b) performing a restriction digest of the DNA with a panel of restriction enzymes; (c) electrophoretically separating the resulting DNA fragments on a sizing gel; (d) contacting the resulting gel with a nucleic acid probe capable of specifically hybridizing with a unique sequence included within the sequence of a nucleic acid molecule encoding a mammalian SNORF33 receptor and labeled with a detectable marker; (e) detecting labeled bands which have hybridized to the DNA encoding a mammalian SNORF33 receptor to create a unique band pattern specific to the DNA of subjects suffering from the disorder; (f) repeating steps (a)-(e) with DNA obtained for diagnosis from subjects not yet suffering from the

disorder; and (g) comparing the unique band pattern specific to the DNA of subjects suffering from the disorder from step (e) with the band pattern from step (f) for subjects not yet suffering from the disorder so as to determine whether the patterns are the same or different and thereby diagnose predisposition to the disorder if the patterns are the same.

In one embodiment, the disorder is a disorder associated with the activity of a specific mammalian allele is diagnosed.

This invention also provides a method of preparing a purified mammalian SNORF33 receptor according to this invention which comprises: (a) culturing cells which express the mammalian SNORF33 receptor; (b) recovering the mammalian SNORF33 receptor from the cells; and (c) purifying the mammalian SNORF33 receptor so recovered.

This invention further provides a method of preparing a purified mammalian SNORF33 receptor according to this invention which comprises: (a) inserting a nucleic acid encoding the mammalian SNORF33 receptor into a suitable expression vector; (b) introducing the resulting vector into a suitable host cell; (c) placing the resulting host cell in suitable condition permitting the production of the mammalian SNORF33 receptor; (d) recovering the mammalian SNORF33 receptor so produced; and optionally (e) isolating and/or purifying the mammalian SNORF33 receptor so recovered.

Furthermore, this invention provides a process for determining whether a chemical compound is a mammalian SNORF33 receptor agonist which comprises contacting cells transfected with and expressing DNA encoding the mammalian SNORF33 receptor with the compound under conditions permitting the activation of the mammalian SNORF33 receptor, and detecting any increase in mammalian SNORF33 receptor activity, so as to thereby determine

whether the compound is a mammalian SNORF33 receptor agonist.

This invention also provides a process for determining whether a chemical compound is a mammalian SNORF33 receptor antagonist which comprises contacting cells transfected with and expressing DNA encoding the mammalian SNORF33 receptor with the compound in the presence of a known mammalian SNORF33 receptor agonist, under conditions permitting the activation of the mammalian SNORF33 receptor, and detecting any decrease in mammalian SNORF33 receptor activity, so as to thereby determine whether the compound is a mammalian SNORF33 receptor antagonist.

In one embodiment, the mammalian SNORF33 receptor is a human SNORF33 receptor. In another embodiment, the mammalian SNORF33 receptor is a rat SNORF33 receptor.

This invention still further provides a composition, for example a pharmaceutical composition, which comprises an amount of a mammalian SNORF33 receptor agonist determined by a process according to this invention effective to increase activity of a mammalian SNORF33 receptor and a carrier, for example, a pharmaceutically acceptable carrier. In one embodiment, the mammalian SNORF33 receptor agonist is not previously known.

Also, this invention provides a composition, for example a pharmaceutical composition, which comprises an amount of a mammalian SNORF33 receptor antagonist determined by a process according to this invention effective to reduce activity of a mammalian SNORF33 receptor and a carrier, for example, a pharmaceutically acceptable carrier. In one embodiment, the mammalian SNORF33 receptor antagonist is not previously known.

This invention moreover provides a process for determining whether a chemical compound specifically binds to and activates a mammalian SNORF33 receptor, which comprises contacting cells

producing a second messenger response and expressing on their cell surface the mammalian SNORF33 receptor, wherein such cells do not normally express the mammalian SNORF33 receptor, with the chemical compound under conditions suitable for activation of the mammalian SNORF33 receptor, and measuring the second messenger response in the presence and in the absence of the chemical compound, a change, e.g. an increase, in the second messenger response in the presence of the chemical compound indicating that the compound activates the mammalian SNORF33 receptor.

In one embodiment, the second messenger response comprises chloride channel activation and the change in second messenger is an increase in the level of chloride current. In another embodiment, the second messenger response comprises change in intracellular calcium levels and the change in second messenger is an increase in the measure of intracellular calcium. In another embodiment, the second messenger response comprises release of inositol phosphate and the change in second messenger is an increase in the level of inositol phosphate. In another embodiment, the second messenger response comprises release of arachidonic acid and the change in second messenger is an increase in the level of arachidonic acid. In yet another embodiment, the second messenger response comprises GTPγS ligand binding and the change in second messenger is an increase in GTPγS ligand binding. In another embodiment, the second messenger response comprises activation of MAP kinase and the change in second messenger response is an increase in MAP kinase activation. In a further embodiment, the second messenger response comprises cAMP accumulation and the change in second messenger response is a reduction in cAMP accumulation.

This invention still further provides a process for determining whether a chemical compound specifically binds to and inhibits activation of a mammalian SNORF33 receptor, which comprises

separately contacting cells producing a second messenger response and expressing on their cell surface the mammalian SNORF33 receptor, wherein such cells do not normally express the mammalian SNORF33 receptor, with both the chemical compound and a second chemical compound known to activate the mammalian SNORF33 receptor, and with only the second chemical compound, under conditions suitable for activation of the mammalian SNORF33 receptor, and measuring the second messenger response in the presence of only the second chemical compound and in the presence of both the second chemical compound and the chemical compound, a smaller change, e.g. increase, in the second messenger response in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound indicating that the chemical compound inhibits activation of the mammalian SNORF33 receptor.

In an embodiment of the present invention, the second chemical compound is a trace amine. Examples of trace amines include, but are not limited to, tryptamine (TYR), tyramine (T), octopamine (OCT), and β-phenyl-ethylamine (PEA).

In one embodiment, the second messenger response comprises chloride channel activation and the change in second messenger response is a smaller increase in the level of chloride current in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound. In another embodiment, the second messenger response comprises change in intracellular calcium levels and the change in second messenger response is a smaller increase in the measure of intracellular calcium in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound. In another embodiment, the second messenger response comprises release of inositol phosphate and the change in second messenger response

is a smaller increase in the level of inositol phosphate in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound.

In one embodiment, the second messenger response comprises activation of MAP kinase and the change in second messenger response is a smaller increase in the level of MAP kinase activation in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound. In another embodiment, the second messenger response comprises change in cAMP levels and the change in second messenger response is a smaller change in the level of cAMP in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound. In another embodiment, the second messenger response comprises release of arachidonic acid and the change in second messenger response is an increase in the level of arachidonic acid levels in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound. In a further embodiment, the second messenger response comprises GTPγS ligand binding and the change in second messenger is a smaller increase in GTPγS ligand binding in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound.

In one embodiment, the mammalian SNORF33 receptor is a human SNORF33 receptor. In a further embodiment, the mammalian SNORF33 receptor is a rat SNORF33 receptor. In another embodiment, the cell is an insect cell. In another embodiment, the cell is a mammalian cell. In another embodiment, the mammalian cell is nonneuronal in origin. In another embodiment, the nonneuronal cell is a COS-7 cell, CHO cell, 293 human embryonic kidney cell,

NIH-3T3 cell or LM(tk-) cell. In another embodiment, the compound is not previously known to bind to a mammalian SNORF33 receptor.

Further, this invention provides a compound determined by a process according to this invention and a composition, for example, a pharmaceutical composition, which comprises an amount of a mammalian SNORF33 receptor agonist determined to be such by a process according to this invention effective to increase activity of the mammalian SNORF33 receptor and a carrier, for example, a pharmaceutically acceptable carrier. In one embodiment, the mammalian SNORF33 receptor agonist is not previously known.

This invention also provides a composition, for example, a pharmaceutical composition, which comprises an amount of a mammalian SNORF33 receptor antagonist determined to be such by a process according to this invention, effective to reduce activity of the mammalian SNORF33 receptor and a carrier, for example a pharmaceutically acceptable carrier. In one embodiment, the mammalian SNORF33 receptor antagonist is not previously known.

This invention yet further provides a method of screening a plurality of chemical compounds not known to activate a mammalian SNORF33 receptor to identify a compound which activates the mammalian SNORF33 receptor which comprises: (a) contacting cells transfected with and expressing the mammalian SNORF33 receptor with the plurality of compounds not known to activate the mammalian SNORF33 receptor, under conditions permitting activation of the mammalian SNORF33 receptor; (b) determining whether the activity of the mammalian SNORF33 receptor is increased in the presence of one or more of the compounds; and if so (c) separately determining whether the activation of the mammalian SNORF33 receptor is increased by any compound included

in the plurality of compounds, so as to thereby identify each compound which activates the mammalian SNORF33 receptor. In one embodiment, the mammalian SNORF33 receptor is a human SNORF33 receptor. In a further embodiment, the mammalian SNORF33 receptor is a rat SNORF33 receptor.

This invention provides a method of screening a plurality of chemical compounds not known to inhibit the activation of a mammalian SNORF33 receptor to identify a compound which inhibits the activation of the mammalian SNORF33 receptor, which comprises: (a) contacting cells transfected with and expressing the mammalian SNORF33 receptor with the plurality of compounds in the presence of a known mammalian SNORF33 receptor agonist, under conditions permitting activation of the mammalian SNORF33 receptor; (b) determining whether the extent or amount of activation of the mammalian SNORF33 receptor is reduced in the presence of one or more of the compounds, relative to the extent or amount of activation of the mammalian SNORF33 receptor in the absence of such one or more compounds; and if so (c) separately determining whether each such compound inhibits activation of the mammalian SNORF33 receptor for each compound included in the plurality of compounds, so as to thereby identify any compound included in such plurality of compounds which inhibits the activation of the mammalian SNORF33 receptor.

In one embodiment, the mammalian SNORF33 receptor is a human SNORF33 receptor. In a further embodiment, the mammalian SNORF33 receptor is a rat SNORF33 receptor. In another embodiment, wherein the cell is a mammalian cell. In another embodiment, the mammalian cell is non-neuronal in origin. In another embodiment, the non-neuronal cell is a COS-7 cell, a 293 human embryonic kidney cell, a LM(tk-) cell or an NIH-3T3 cell.

This invention also provides a composition, for example, a pharmaceutical composition, comprising a compound identified by

a method according to this invention in an amount effective to increase mammalian SNORF33 receptor activity and a carrier, for example, a pharmaceutically acceptable carrier.

This invention still further provides a composition, for example, a pharmaceutical composition, comprising a compound identified by a method according to this invention in an amount effective to decrease mammalian SNORF33 receptor activity and a carrier, for example, a pharmaceutically acceptable carrier.

Furthermore, this invention provides a method of treating an abnormality in a subject wherein the abnormality is alleviated by increasing the activity of a mammalian SNORF33 receptor which comprises administering to the subject a compound which is a mammalian SNORF33 receptor agonist in an amount effective to treat the abnormality. In one embodiment, the abnormality is a regulation of a steroid hormone disorder, an epinephrine release disorder, a gastrointestinal disorder, a cardiovascular disorder, an electrolyte balance disorder, hypertension, diabetes, a respiratory disorder, asthma, a reproductive function disorder, an immune disorder, an endocrine disorder, a musculoskeletal disorder, a neuroendocrine disorder, a cognitive disorder, a memory disorder, somatosensory and neurotransmission disorders, a motor coordination disorder, a sensory integration disorder, a motor integration disorder, a dopaminergic function disorder, an appetite disorder, such as anorexia or obesity, a sensory transmission disorder, an olfaction disorder, an autonomic nervous system disorder, pain, psychotic behavior, affective disorder, migraine, circadian disorders, visual disorders, urinary disorders, blood coagulation-related disorders, developmental disorders, or ischemia-reperfusion injury-related diseases.

This invention additionally provides a method of treating an abnormality in a subject wherein the abnormality is alleviated

by decreasing the activity of a mammalian SNORF33 receptor which comprises administering to the subject a compound which is a mammalian SNORF33 receptor antagonist in an amount effective to treat the abnormality. In one embodiment, the abnormality is a regulation of a steroid hormone disorder, an epinephrine release disorder, a gastrointestinal disorder, a cardiovascular disorder, an electrolyte balance disorder, hypertension, diabetes, a respiratory disorder, asthma, a reproductive function disorder, an immune disorder, an endocrine disorder, a musculoskeletal disorder, a neuroendocrine disorder, a cognitive disorder, a memory disorder, somatosensory and neurotransmission disorders, a motor coordination disorder, a sensory integration disorder, a motor integration disorder, a dopaminergic function disorder, an appetite disorder, such as anorexia or obesity, a sensory transmission disorder, an olfaction disorder, an autonomic nervous system disorder, pain, psychotic behavior, affective disorder, migraine, circadian disorders, visual disorders, urinary disorders, blood coagulation-related disorders, developmental disorders, or ischemia-reperfusion injury-related diseases.

This invention also provides a process for making a composition of matter which specifically binds to a mammalian SNORF33 receptor which comprises identifying a chemical compound using a process in accordance with this invention and then synthesizing the chemical compound or a novel structural and functional analog or homolog thereof. In one embodiment, the mammalian SNORF33 receptor is a human SNORF33 receptor. In another embodiment, the mammalian SNORF33 receptor is a rat SNORF33 receptor.

This invention further provides a process for preparing a composition, for example a pharmaceutical composition which comprises admixing a carrier, for example, a pharmaceutically acceptable carrier, and a pharmaceutically effective amount of a chemical compound identified by a process in accordance with

this invention or a novel structural and functional analog or homolog thereof. In one embodiment, the mammalian SNORF33 receptor is a human SNORF33 receptor. In another embodiment, the mammalian SNORF33 receptor is a rat SNORF33 receptor.

Thus, once the gene for a targeted receptor subtype is cloned, it is placed into a recipient cell which then expresses the targeted receptor subtype on its surface. This cell, which expresses a single population of the targeted human receptor subtype, is then propagated resulting in the establishment of a cell line. This cell line, which constitutes a drug discovery system, is used in two different types of assays: binding assays and functional assays. In binding assays, the affinity of a compound for both the receptor subtype that is the target of a particular drug discovery program and other receptor subtypes that could be associated with side effects are measured. These measurements enable one to predict the potency of a compound, as well as the degree of selectivity that the compound has for the targeted receptor subtype over other receptor subtypes. The data obtained from binding assays also enable chemists to design compounds toward or away from one or more of the relevant subtypes, as appropriate, for optimal therapeutic efficacy. In functional assays, the nature of the response of the receptor subtype to the compound is determined. Data from the functional assays show whether the compound is acting to inhibit or enhance the activity of the receptor subtype, thus enabling pharmacologists to evaluate compounds rapidly at their ultimate human receptor subtypes targets permitting chemists to rationally design drugs that will be more effective and have fewer or substantially less severe side effects than existing drugs.

Approaches to designing and synthesizing receptor subtype-selective compounds are well known and include traditional medicinal chemistry and the newer technology of combinatorial chemistry, both of which are supported by computer-assisted

molecular modeling. With such approaches, chemists and pharmacologists use their knowledge of the structures of the targeted receptor subtype and compounds determined to bind and/or activate or inhibit activation of the receptor subtype to design and synthesize structures that will have activity at these receptor subtypes.

Combinatorial chemistry involves automated synthesis of a variety of novel compounds by assembling them using different combinations of chemical building blocks. The use of combinatorial chemistry greatly accelerates the process of generating compounds. The resulting arrays of compounds are called libraries and are used to screen for compounds ("lead compounds") that demonstrate a sufficient level of activity at receptors of interest. Using combinatorial chemistry it is possible to synthesize "focused" libraries of compounds anticipated to be highly biased toward the receptor target of interest.

Once lead compounds are identified, whether through the use of combinatorial chemistry or traditional medicinal chemistry or otherwise, a variety of homologs and analogs are prepared to facilitate an understanding of the relationship between chemical structure and biological or functional activity. These studies define structure activity relationships which are then used to design drugs with improved potency, selectivity and pharmacokinetic properties. Combinatorial chemistry is also used to rapidly generate a variety of structures for lead optimization. Traditional medicinal chemistry, which involves the synthesis of compounds one at a time, is also used for further refinement and to generate compounds not accessible by automated techniques. Once such drugs are defined the production is scaled up using standard chemical manufacturing methodologies utilized throughout the pharmaceutical and chemistry industry.

This invention will be better understood from the Experimental Details which follow. However, one skilled in the art will readily appreciate that the specific methods and results discussed are merely illustrative of the invention as described more fully in the claims which follow thereafter.

EXPERIMENTAL DETAILS

Materials and Methods

MOPAC (Mixed Oligonucleotide Primed Amplification of cDNA)

100 ng of rat genomic DNA (Clontech, Palo Alto, CA) was used for degenerate MOPAC PCR using Taq DNA polymerase (Boehringer-Mannheim, Indianapolis, IN) and the following degenerate oligonucleotides: BB726, designed based on an alignment of the sixth transmembrane domain of select serotonin (5-HT) receptors; and BB642, designed based on an alignment of the seventh transmembrane domain of the same serotonin receptors.

The conditions for the MOPAC PCR reaction were as follows: 5 minute hold at 94°C; 10 cycles of 30 seconds at 94°C, 1 minute at 43°C, 1 minute 45 seconds at 72°C ; 30 cycles of 30 seconds at 94°C, 1 minute at 48°C, 1 minute 45 seconds at 72°C; 20 minute hold at 72°C; 4°C hold until ready for agarose gel electrophoresis.

The products were run on a 1.5% agarose TAE gel and bands of the expected size (~150 bp) were cut from the gel, purified using the QIAQUICK gel extraction kit (QIAGEN, Chatsworth, CA), and subcloned into the TA cloning vector (Invitrogen, San Diego, CA). White (insert-containing) colonies were picked and subjected to PCR using pCR2.1 vector primers JAB1 and JAB2 using the Expand Long Template PCR System and the following protocol: 94°C hold for 3 minutes; 35 cycles of 94°C for 1 minute, 68°C for 1 minute 15 seconds; 2 minute hold at 68°C, 4°C hold until the products were ready for purification. PCR products were purified by isopropanol precipitation (10 μl PCR product, 18 μl low TE, 10.5 μl 2M $NaClO_4$, and 21.5 μl isopropanol) and sequenced using the ABI Big Dye cycle sequencing protocol and ABI 377 sequencers (ABI, Foster City, CA). Nucleotide and amino acid sequence

analyses were performed using the Wisconsin Package (GCG, Genetics Computer Group, Madison, WI). One PCR product from rat genomic DNA (5-HT-38-rgen-051) was determined to be a novel G protein-coupled receptor-like sequence based on database searches and its homology to other known G protein-coupled receptors (~42-48% amino acid identity to $5HT_4$, dopamine D_2 and β-adrenergic receptors). This novel sequence was designated SNORF33.

<u>Cloning of the full-length coding sequence of rat SNORF33</u>

A rat liver genomic phage library (2.75 million recombinants, Stratagene, LaJolla, CA) was screened using a ^{32}P-labeled oligonucleotide probe, HK132, designed against the rat SNORF33 fragment.

Hybridization of nitrocellulose filter overlays of the plates was performed at high stringency: 42°C in a solution containing 50% formamide, 5x SSC (1X SSC is 0.15M sodium chloride, 0.015M sodium citrate), 1x Denhardt's solution (0.02% polyvinylpyrrolindone, 0.02% Ficoll, 0.02% bovine serum albumin), 7 mM Tris and 25 μg/ml sonicated salmon sperm DNA. The filters were washed at 55°C in 0.1x SSC containing 0.1% sodium dodecyl sulfate and exposed at -70°C to Kodak BioMax MS film in the presence of an intensifying screen.

A positive signal on plate 35 was isolated on a tertiary plating. A 5.5 kb fragment, from a *Bgl*II digest of DNA isolated from this positive, was identified by Southern blot analysis, subcloned into pcDNA3.1 (Invitrogen, San Diego, CA) and used to transform E.coli DH5α cells (Gibco BRL, Gaithersburg MD). Plasmid DNA from one transformant, KO26, was sequenced on both strands using an ABI 377 sequencer as described above. Sequencing of KO26 revealed an open reading frame of 996 nucleotides with approximately 3.7 kb of upstream sequence and 0.8 kb of

downstream sequence. A 1.8 kb *EcoRI-HindIII* fragment from KO26 was subcloned into pcDNA3.1. This construct, designated BO111, contains the 996 bp coding region as well as 81 nucleotides of 5′ untranslated region and 791 bp of 3′ untranslated region. This construct, BO111, has been renamed pcDNA3.1-rSNORF33-f. The full length SNORF33 was determined to have significant homology with PNR (38% amino acid identity), $5HT_{1D}$, $5HT_4$ and dopamine D_1 receptors (35-36% amino acid identities) and histamine H_1 and α_{1c} adrenergic receptors (33% amino acid identity). There were no sequences in the Genbank databases (Genembl, STS, EST, GSS, or SwissProt) that were identical to SNORF33.

Isolation of a Fragment of the Human Homologue of SNORF33

To obtain a fragment of the human homologue of SNORF33, 100 ng of human genomic DNA (Clontech, Palo Alto, CA) was amplified with a forward PCR primer corresponding to TMI of the rat SNORF33 (BB990) and a reverse primer corresponding to TMVII of the rat SNORF33 (BB991). PCR was performed with the Expand Long Template PCR System (Boeringer Mannheim) under the following conditions: 30 seconds at 94°C, 1 minute at 47°C or 51°C, 1.5 minutes at 68°C for 40 cycles, with a pre- and post-incubation of 5 minutes at 94°C and 7 minutes at 68°C respectively. Bands of 833 bp from 2 independent PCR reactions were isolated from a TAE gel, purified using the QIAQUICK gel extraction kit (QIAGEN, Chatsworth, CA), and sequenced on both strands as described above. The sequence of these two PCR products were identical and were used to design forward and reverse PCR primers (BB997, also incorporating a *Bam*HI restriction site, and BB998, also incorporating a *Hind*III site) which were used to amplify a band from human genomic DNA using the following conditions: 30 seconds at 94°C, 2 minute at 68°C for 40 cycles, with a pre- and post-incubation of 5 minutes at 94°C and 7 minutes at 68°C, respectively. Products from 6 independent PCR reactions were digested with *EcoRI* and *BamHI*, and

fragments of 590 bp were gel-purified and ligated into pcDNA3.1 (Invitrogen, San Diego, CA). One transformant from each PCR reaction was sequenced as above, and a consensus sequence determined. The nucleotide sequence of one product, KO28, was identical to the consensus. This construct has been renamed pcDNA3.1-hSNORF33-p.

Isolation of the full-length human SNORF33 receptor cDNA

A nucleic acid sequence encoding a human SNORF33 receptor cDNA may be isolated using standard molecular biology techniques and approaches such as those described below:

Approach #1: A human genomic library (e.g., cosmid, phage, P1, BAC, YAC) may be screened with a ^{32}P-labeled oligonucleotide probe corresponding to the human fragment whose sequence is shown in Figure 1 to isolate a genomic clone. The full-length sequence may be obtained by sequencing this genomic clone. If one or more introns are present in the gene, the full-length intronless gene may be obtained from cDNA using standard molecular biology techniques. For example, a forward PCR primer designed in the 5'UT and a reverse PCR primer designed in the 3'UT may be used to amplify a full-length, intronless receptor from cDNA. Standard molecular biology techniques could be used to subclone this gene into a mammalian expression vector.

Approach #2: Standard molecular biology techniques may be used to screen commercial cDNA phage libraries by hybridization under high stringency with a ^{32}P-labeled oligonucleotide probe corresponding to the human fragment whose sequence is shown in Figure 1. One may isolate a full-length human SNORF33 receptor by obtaining a plaque purified clone from the lambda libraries and then subjecting the clone to direct DNA sequencing. Alternatively, standard molecular biology techniques could be used to screen human cDNA plasmid libraries by PCR amplification

of library pools using primers designed against the partial human sequence. A full-length clone may be isolated by Southern hybridization of colony lifts of positive pools with a ^{32}P-oligonucleotide probe.

Approach #3: 3' and 5' RACE may be utilized to generate PCR products from cDNA expressing SNORF33 which contain the additional sequence of SNORF33. These RACE PCR products may then be sequenced to determine the additional sequence. This new sequence is then used to design a forward PCR primer in the 5'UT and a reverse primer in the 3'UT. These primers are then used to amplify a full-length SNORF33 clone from cDNA.

Cloning of the full-length human SNORF33

5' and 3' RACE

To isolate the full-length human SNORF33, we chose approach #3 described above. Specifically, we utilized the Clontech Marathon cDNA Amplification kit (Clontech, Palo Alto, CA) for 5'/3' Rapid Amplification of cDNA ends (RACE). Nested PCR were performed according to the Marathon cDNA Amplification protocol using Marathon-Ready human kidney and stomach cDNA (Clontech). For 5'RACE, the initial PCR was performed with the supplier's Adapter Primer 1 and BB1049, a reverse primer from TMIII of the PCR fragment described above. One ul of this initial PCR reaction was re-amplified using the Adaptor Primer 2 and BB1021, a reverse primer from TMII. PCR was performed with Advantage Klentaq Polymerase (Clontech, Palo Alto, CA) under the following conditions: 5 minutes at 94°C; 5 cycles of 94°C for 30 seconds and 72°C (initial PCR) or 70°C (nested PCR) for 2 minutes; 5 cycles of 94°C for 30 seconds and 70°C (initial PCR) or 68°C (nested PCR) for 2 minutes; 25 cycles (initial PCR) or 18 cycles (nested PCR) of 94°C for 30 seconds and 68°C (initial PCR) or 66°C (nested PCR) for 2 minutes; 68°C hold for 7 minutes, and 4°C hold until the products were ready for analysis. For 3'RACE, the initial PCR was performed with the supplier's Adapter Primer 1 and BB1050,

a forward primer from the V-VI loop of the PCR fragment described above. Two uls of this initial PCR reaction was re-amplified using the Adaptor Primer 2 and BB1022, a forward PCR primer from TMVI.

PCR was performed with the Expand Long Template PCR System (Roche Molecular Biochemicals, Indianapolis, Indiana) under the following conditions: 5 minutes at 94° C; 5 cycles of 94° C for 30 seconds, 72° C (initial PCR) or 70° C (nested PCR) for 45 seconds, 68 ° C for 2 minutes; 5 cycles of 94° C for 30 seconds, 70° C (initial PCR) or 68° C (nested PCR) for 45 seconds and 68 ° C for 2 minutes; 25 cycles (initial PCR) or 18 cycles (nested PCR) of 94° C for 30 seconds and 68° C (initial PCR) or 66° C (nested PCR) for 45 seconds and 68 ° C for 2 minutes; 68° C hold for 7 minutes, and 4° C hold until the products were ready for analysis. A 300 bp and a 500 bp fragment from the 5′ RACE and a 350 bp fragment from the 3′ RACE were isolated from a 1% agarose TAE gel using the QIAQUICK kit and sequenced using ABI 377 sequencers and BigDye termination cycle sequencing as described above. Sequences were analyzed using the Wisconsin Package (GCG, Genetics Computer Group, Madison, WI.).

Isolation of a full-length human SNORF33 clone

After determining the full-length coding sequence of this receptor sequence, the entire coding region was amplified from human genomic DNA and human amygdala cDNA using the Expand Long Template PCR system (Roche Molecular Biochemicals, Indianapolis, Indiana). The primers for this reaction were specific to the 5′ and 3′ untranslated regions of SNORF33 with *BamHI* and *Hind*III restriction sites incorporated into the 5′ ends of the 5′ (BB1101) and 3′ (BB1102) primers, respectively. The products from 7 independent PCR reactions were then digested with *BamHI* and *Hind*III, subcloned into the *BamHI* and *Hind*III sites of the expression vector pcDNA3.1 (-), and sequenced in both directions using vector- and gene-specific primers. One construct, GEN-p1c4, matched the consensus and was renamed BO113. This receptor/expression vector construct of human SNORF33 in pcDNA3.1(-)

was named pcDNA3.1-hSNORF33-f. A *BamHI/HindIII* fragment of BO113, containing the entire SNORF33 insert, was ligated into *BamHI/HindIII* digested pEXJ.RHT3T7 vector. This construct, BO114, was named pEXJ-hSNORF33-f.

Isolation of a Fragment of the Mouse Homologue of SNORF33

To obtain a fragment of the mouse homologue of SNORF33, 100 ng of mouse genomic DNA (Clontech, Palo Alto, CA) was amplified with a forward PCR primer corresponding to TMI of the rat SNORF33 (BB982) and a reverse primer corresponding to TMVII of the rat SNORF33 (BB983). PCR was performed with the Expand Long Template PCR System (Boeringer Mannheim) under the following conditions: 30 seconds at 94 ° C, 45 seconds at 45 to 51 ° C, 2 minutes at 68 ° C for 37 cycles, with a pre- and post-incubation of 5 minutes at 95 ° C and 7 minutes at 68 ° C respectively. Bands of 800 bp from 7 independent PCR reactions were isolated from a TAE gel, purified using the QIAQUICK gel extraction kit (QIAGEN, Chatsworth, CA), and sequenced on both strands as described above. A consensus sequence was determined for these seven products, and was used to design forward and reverse PCR primers (BB1273, also incorporating a *BamHI* restriction site, and BB1274, also incorporating a *HindIII* site) which were used to amplify a band from mouse genomic DNA using the following conditions: 30 seconds at 94 ° C, 1.5 minutes at 68 ° C for 32 cycles, with a pre- and post-incubation of 5 minutes at 94 ° C and 7 minutes at 68 ° C, respectively. Products from 4 independent PCR reactions were digested with *BamHI* and *HindIII*, and fragments of 252 bp were gel-purified and ligated into pEXJ.T3T7. One transformant from each PCR reaction was sequenced as above, and all four sequences were determined to be identical. The nucleotide and amino acid sequences of one product, KO94, are shown in Figures 17 and 18, respectively. KO94 was renamed pEXJ.T3T7-mSNORF33-p.

It is anticipated that a molecular biologist skilled in the art may isolate the full-length mouse SNORF33 receptor using standard molecular biology techniques and approaches such as those briefly described below:

Approach #1: Pools of in-house mouse cDNA plasmid libraries may be screened by high stringency PCR with primers designed against the mouse SNORF33 partial sequence. Positive pools could be sib-selected and then colonies from a low complexity subpool could be screened by filter hybridization using an oligonucleotide probe designed against the mouse SNORF33 fragment.

Approach #2: Standard molecular biology techniques could be used to screen commercial phage cDNA or genomic libraries by filter hybridization under high stringency conditions using an oligonucleotide probe designed against the mouse SNORF33 fragment.

Approach #3: As yet another alternative method, one could utilize 5′ and 3′ RACE to generate PCR products from mouse cDNA expressing mouse SNORF33 which would contain the additional 5′ and 3′ sequences of this receptor. For example, Marathon-Ready cDNA (Clontech, Palo Alto, CA) could be used as instructed by the manufacturer. Nested reverse PCR primers designed against the mouse SNORF33 fragment could be used for 5′ RACE and nested forward PCR primers could be used for 3′ RACE. Using this new sequence, a forward PCR primer designed in the 5′untranslated region and a reverse PCR primer designed in the 3′untranslated region could be used to amplify a full-length SNORF33 receptor cDNA from either genomic DNA or mouse tissue cDNA.

Oligonucleotide primers and probes used in the identification and isolation of SNORF33:

JAB1: 5′-TTATGCTTCCGGCTCGTATGTTGTG-3′ (SEQ ID No: 7)

JAB2: 5'-ATGTGCTGCAAGGCGATTAAGTTGGG-3' (SEQ ID No: 8)
BB726 5'-TNNKNTGYTGGYTNCCNTTYTTY-3' (SEQ ID No: 9)
BB642 5'-ARNSWRTTNVNRTANCCNARCC-3' (SEQ ID No: 10)
HK132 5'-TTCTGCATGGTCCTGGACCCTTTCCTGGGCTATGTTATCCCACCCACT CTGAATGACACACTG-3' (SEQ ID No: 11)
BB990 5'-CATAATTCTAACCACTCTGGTTGG-3' (SEQ ID No: 12)
BB991 5'-CTGAACCAGGGATAGAAAAAGGC-3' (SEQ ID No: 13)
BB997 5'-TCCGTAGGATCCAATTGGCTCATTCATTCCATGGCC-3' (SEQ ID No: 14)
BB998 5'-AGCTACAAGCTTGCACCAGCATATTAGGAAAACTCC-3' (SEQ ID No: 15)
BB1049 5'-CAGCATAATGTCGGTGCTTGTGTG-3' (SEQ ID No: 16)
BB1021 5'-TACTGTAAGGCATGACCAGACACC-3' (SEQ ID No: 17)
BB1050 5'-ATTAGTGATGCCAATCAGAAGCTCC-3' (SEQ ID No: 18)
BB1022 5'-GAAAGGAAAGCTGTGAAGACATTGG-3' (SEQ ID No: 19)
BB1101 5'-GATCTAGGATCCGGAAAAGTAAACTGATTGACAGCCC-3' (SEQ ID No: 20)
BB1102 5'-CTAGCTAAGCTTGATCATCAACCGATTTGCAAAACAG-3' (SEQ ID No: 21)
BB982 5'-ACTCTGGTTGGCAACTTAATAGT-3' (SEQ ID No: 32)
BB983 5'-GCATAAACCATCGGGTTGAAGGC-3' (SEQ ID No: 33)
BB1273 5'-TATCGCGGATCCGGTACTGGCGTTCATGACTTCCTTC-3' (SEQ ID No: 34)
BB1274 5'-CCAGCTAAGCTTAGGAAAGGGTCCAGGACCGTGCAG-3' (SEQ ID No: 35)

Isolation of other species homologs of SNORF33 receptor cDNA

A nucleic acid sequence encoding a SNORF33 receptor cDNA from other species may be isolated using standard molecular biology techniques and approaches such as those described below:

Approach #1: A genomic library (e.g., cosmid, phage, P1, BAC, YAC) generated from the species of interest may be screened with a ^{32}P-labeled oligonucleotide probe corresponding to a fragment of the human or rat SNORF33 receptors whose sequence is shown in Figures 1, 3A-3B and 5A-5B to isolate a genomic clone. The full-length sequence may be obtained by sequencing this genomic clone.

If one or more introns are present in the gene, the full-length intronless gene may be obtained from cDNA using standard molecular biology techniques. For example, a forward PCR primer designed in the 5'UT and a reverse PCR primer designed in the 3'UT may be used to amplify a full-length, intronless receptor from cDNA. Standard molecular biology techniques could be used to subclone this gene into a mammalian expression vector.

Approach #2: Standard molecular biology techniques may be used to screen commercial cDNA phage libraries of the species of interest by hybridization under reduced stringency with a ^{32}P-labeled oligonucleotide probe corresponding to a fragment of the sequences shown in Figures 1, 3A-3B, or 5A-5B. One may isolate a full-length SNORF33 receptor by obtaining a plaque purified clone from the lambda libraries and then subjecting the clone to direct DNA sequencing. Alternatively, standard molecular biology techniques could be used to screen cDNA plasmid libraries by PCR amplification of library pools using primers designed against a partial species homolog sequence. A full-length clone may be isolated by Southern hybridization of colony lifts of positive pools with a ^{32}P-oligonucleotide probe.

Approach #3: 3' and 5' RACE may be utilized to generate PCR products from cDNA derived from the species of interest expressing SNORF33 which contain the additional sequence of SNORF33. These RACE PCR products may then be sequenced to determine the additional sequence. This new sequence is then used to design a forward PCR primer in the 5'UT and a reverse primer in the 3'UT. These primers are then used to amplify a full-length SNORF33 clone from cDNA.

Examples of other species include, but are not limited to, dog, monkey, hamster and guinea pig.

Host cells

A broad variety of host cells can be used to study heterologously expressed proteins. These cells include but are not limited to mammalian cell lines such as; COS-7, CHO, LM*(tk⁻)*, HEK293, etc.; insect cell lines such as; Sf9, Sf21, etc.; amphibian cells such as *Xenopus* oocytes; assorted yeast strains; assorted bacterial cell strains; and others. Culture conditions for each of these cell types is specific and is known to those familiar with the art.

Transient expression

DNA encoding proteins to be studied can be transiently expressed in a variety of mammalian, insect, amphibian, yeast, bacterial and other cells lines by several transfection methods including but not limited to; calcium phosphate-mediated, DEAE-dextran mediated; liposomal-mediated, viral-mediated, electroporation-mediated, and microinjection delivery. Each of these methods may require optimization of assorted experimental parameters depending on the DNA, cell line, and the type of assay to be subsequently employed.

Stable expression

Heterologous DNA can be stably incorporated into host cells, causing the cell to perpetually express a foreign protein. Methods for the delivery of the DNA into the cell are similar to those described above for transient expression but require the co-transfection of an ancillary gene to confer drug resistance on the targeted host cell. The ensuing drug resistance can be exploited to select and maintain cells that have taken up the DNA. An assortment of resistance genes are available including but not restricted to neomycin, kanamycin, and hygromycin. For the purposes of studies concerning the receptor of this invention, stable expression of a heterologous receptor protein is typically carried out in, mammalian cells including but not necessarily restricted to, CHO, HEK293, LM(tk-), etc.

In addition native cell lines that naturally carry and express the nucleic acid sequences for the receptor may be used without the need to engineer the receptor complement.

Functional assays

Cells expressing the receptor DNA of this invention may be used to screen for ligands to said receptor using functional assays. Once a ligand is identified the same assays may be used to identify agonists or antagonists of the receptor that may be employed for a variety of therapeutic purposes. It is well known to those in the art that the over-expression of a G-protein coupled receptor can result in the constitutive activation of intracellular signaling pathways. In the same manner, over-expression of the receptor in any cell line as described above, can result in the activation of the functional responses described below, and any of the assays herein described can be used to screen for agonist, partial agonist, inverse agonist, and antagonist ligands of the SNORF33 receptor.

A wide spectrum of assays can be employed to screen for the presence of receptor rSNORF33 ligands. These assays range from traditional measurements of total inositol phosphate accumulation, cAMP levels, intracellular calcium mobilization, and potassium currents, for example; to systems measuring these same second messengers but which have been modified or adapted to be of higher throughput, more generic and more sensitive; to cell based assays reporting more general cellular events resulting from receptor activation such as metabolic changes, differentiation, cell division/proliferation. Description of several such assays follow.

Cyclic AMP (cAMP) assay

The receptor-mediated stimulation or inhibition of cyclic AMP (cAMP) formation may be assayed in cells expressing the mammalian

receptors. Cells are plated in 96-well plates or other vessels and preincubated in a buffer such as HEPES buffered saline (NaCl (150 mM), $CaCl_2$ (1 mM), KCl (5 mM), glucose (10 mM)) supplemented with a phosphodiesterase inhibitor such as 5mM theophylline, with or without protease inhibitor cocktail (For example, a typical inhibitor cocktail contains 2 µg/ml aprotinin, 0.5 mg/ml leupeptin, and 10 µg/ml phosphoramidon.) for 20 min at 37°C, in 5% CO_2. Test compounds are added with or without 10 mM forskolin and incubated for an additional 10 min at 37°C. The medium is then aspirated and the reaction stopped by the addition of 100 mM HCl or other methods. The plates are stored at 4°C for 15 min, and the cAMP content in the stopping solution is measured by radioimmunoassay. Radioactivity may be quantified using a gamma counter equipped with data reduction software. Specific modifications may be performed to optimize the assay for the receptor or to alter the detection method of cAMP.

COS-7 cells were transiently transfected with rSNORF33 gene using the calcium phosphate method and plated in 96-well plates. 48 hours after transfection, cells were washed twice with Dulbecco's phosphate buffered saline (PBS) supplemented with 10 mM HEPES, 10 mM glucose, 5 mM theophylline and 10 µM pargyline and were incubated in the same buffer for 20 min at 37°C, in 95% O_2 and 5% CO_2. Test compounds were added and cells were incubated for an additional 10 min at 37°C. The medium was then aspirated and the reaction stopped by the addition of 100 mM HCl. The plates were stored at -20°C for 2-5 days. For cAMP measurement, plates were thawed and the cAMP content in each well was measured by radioimmunoassay cAMP Scintillation Proximity Assay (Amersham Pharmacia Biotech). Radioactivity was quantified using microbeta Trilux counter (Wallac).

Arachidonic acid release assay

Cells expressing the receptor are seeded into 96 well plates or

other vessels and grown for 3 days in medium with supplements. ^{3}H-arachidonic acid (specific activity = 0.75 µCi/ml) is delivered as a 100 µL aliquot to each well and samples are incubated at 37°C, 5% CO_2 for 18 hours. The labeled cells are washed three times with medium. The wells are then filled with medium and the assay is initiated with the addition of test compounds or buffer in a total volume of 250 µL. Cells are incubated for 30 min at 37°C, 5% CO_2. Supernatants are transferred to a microtiter plate and evaporated to dryness at 75°C in a vacuum oven. Samples are then dissolved and resuspended in 25 µL distilled water. Scintillant (300 µL) is added to each well and samples are counted for ^{3}H in a Trilux plate reader. Data are analyzed using nonlinear regression and statistical techniques available in the GraphPAD Prism package (San Diego, CA).

Intracellular calcium mobilization assays

The intracellular free calcium concentration may be measured by microspectrofluorimetry using the fluorescent indicator dye Fura-2/AM (Bush et al, 1991). Cells expressing the receptor are seeded onto a 35 mm culture dish containing a glass coverslip insert and allowed to adhere overnight. Cells are then washed with HBS and loaded with 100 µL of Fura-2/AM (10 µM) for 20 to 40 min. After washing with HBS to remove the Fura-2/AM solution, cells are equilibrated in HBS for 10 to 20 min. Cells are then visualized under the 40X objective of a Leitz Fluovert FS microscope and fluorescence emission is determined at 510 nM with excitation wavelengths alternating between 340 nM and 380 nM. Raw fluorescence data are converted to calcium concentrations using standard calcium concentration curves and software analysis techniques.

In another method, the measurement of intracellular calcium can

also be performed on a 96-well (or higher) format and with alternative calcium-sensitive indicators, preferred examples of these are: aequorin, Fluo-3, Fluo-4, Fluo-5, Calcium Green-1, Oregon Green, and 488 BAPTA. After activation of the receptors with agonist ligands the emission elicited by the change of intracellular calcium concentration can be measured by a luminometer, or a fluorescence imager; a preferred example of this is the fluorescence imager plate reader (FLIPR).

Cells expressing the receptor of interest are plated into clear, flat-bottom, black-wall 96-well plates (Costar) at a density of 30,000-80,000 cells per well and allowed to incubate over night at 5% CO_2, 37°C. The growth medium is aspirated and 100 µl of dye loading medium is added to each well. The loading medium contains: Hank's BSS (without phenol red)(Gibco), 20 mM HEPES (Sigma), 0.1% BSA (Sigma), dye/pluronic acid mixture (e.g. 1 mM Flou-3, AM (Molecular Probes), 10% pluronic acid (Molecular Probes); (mixed immediately before use), and 2.5 mM probenecid (Sigma)(prepared fresh)). The cells are allowed to incubate for about 1 hour at 5% CO_2, 37°C.

During the dye loading incubation the compound plate is prepared. The compounds are diluted in wash buffer (Hank's BSS without phenol red), 20 mM HEPES, 2.5 mM probenecid to a 3X final concentration and aliquoted into a clear v-bottom plate (Nunc). Following the incubation the cells are washed to remove the excess dye. A Denley plate washer is used to gently wash the cells 4 times and leave a 100 µl final volume of wash buffer in each well. The cell plate is placed in the center tray and the compound plate is placed in the right tray of the FLIPR. The FLIPR software is setup for the experiment, the experiment is run and the data are collected. The data are then analyzed using an excel spreadsheet program.

Antagonist ligands are identified by the inhibition of the signal elicited by agonist ligands.

In another method, intracellular free calcium concentration may be measured by the fluorescence imager plate reader (FLIPR). Cells expressing the receptor of interest are plated into clear, flat-bottom, black-wall 96-well plates (Costar) at a density of 80,000-150,000 cells per well and allowed to incubate for 48 hr at 95% O_2/5% CO_2, 37°C. The growth medium is aspirated and 100 µl of loading medium containing fluo-3 dye is added to each well. The loading medium contains: Hank's BSS (without phenol red)(Gibco), 20 mM HEPES (Sigma), 0.1 or 1% BSA (Sigma), dye/pluronic acid mixture (e.g. 1 mM Flou-3, AM (Molecular Probes) and 10% pluronic acid (Molecular Probes) mixed immediately before use), and 2.5 mM probenecid (Sigma)(prepared fresh). The cells are allowed to incubate for about 1 hour at 95% O_2/5% CO_2, 37°C.

During the dye loading incubation the compound plate is prepared. The compounds are diluted in wash buffer (Hank's BSS (without phenol red), 20 mM HEPES, 2.5 mM probenecid) to a 4X final concentration and aliquoted into a clear v-bottom plate (Nunc). Following the incubation the cells are washed to remove the excess dye. A Denley plate washer is used to gently wash the cells 4 times and leave a 100 µl final volume of wash buffer in each well. The cell plate is placed in the center tray and the compound plate is placed in the right tray of the FLIPR. The FLIPR software is setup for the experiment, the experiment is run and the data are collected. The data are then analyzed using an excel spreadsheet program.

Inositol phosphate assay

Receptor mediated activation of the inositol phosphate (IP) second messenger pathways may be assessed by radiometric or other

measurement of IP products.

For example, in a 96 well microplate format assay, cells are plated at a density of 70,000 cells per well and allowed to incubate for 24 hours. The cells are then labeled with 0.5 μCi [^{3}H]myo-inositol overnight at 37°C, 5% CO_2. Immediately before the assay, the medium is removed and replaced with 90 μL of PBS containing 10 mM LiCl. The plates are then incubated for 15 min at 37°C, 5% CO_2. Following the incubation, the cells are challenged with agonist (10 ml/well; 10x concentration) for 30 min at 37°C, 5% CO_2. The challenge is terminated by the addition of 100 μL of 50% v/v trichloroacetic acid, followed by incubation at 4°C for greater than 30 minutes. Total IPs are isolated from the lysate by ion exchange chromatography. Briefly, the lysed contents of the wells are transferred to a Multiscreen HV filter plate (Millipore) containing Dowex AG1-X8 (200-400 mesh, formate form). The filter plates are prepared adding 100 μL of Dowex AG1-X8 suspension (50% v/v, water: resin) to each well. The filter plates are placed on a vacuum manifold to wash or elute the resin bed. Each well ~~is~~ first washed 2 times with 200 μl of 5 mM myo-inositol. Total [^{3}H]-inositol phosphate is eluted with 75 μl of 1.2M ammonium formate/0.1M formic acid solution into 96-well plates. 200 μL of scintillation cocktail is added to each well, and the radioactivity is determined by liquid scintillation counting.

GTPγS functional assay

Membranes from cells expressing the receptor are suspended in assay buffer (e.g., 50 mM Tris, 100 mM NaCl, 5 mM $MgCl_2$, 10 μM GDP, pH 7.4) with or without protease inhibitors (e.g., 0.1% bacitracin). Membranes are incubated on ice for 20 minutes, transferred to a 96-well Millipore microtiter GF/C filter plate and mixed with GTPγ^{35}S (e.g., 250,000 cpm/sample, specific

activity ~1000 Ci/mmol) plus or minus unlabeled GTPγS (final concentration = 100 μM). Final membrane protein concentration ≈ 90 μg/ml. Samples are incubated in the presence or absence of test compounds for 30 min. at room temperature, then filtered on a Millipore vacuum manifold and washed three times with cold (4°C) assay buffer. Samples collected in the filter plate are treated with scintillant and counted for ^{35}S in a Trilux (Wallac) liquid scintillation counter. It is expected that optimal results are obtained when the receptor membrane preparation is derived from an appropriately engineered heterologous expression system, i.e., an expression system resulting in high levels of expression of the receptor and/or expressing G-proteins having high turnover rates (for the exchange of GDP for GTP). GTPγS assays are well-known to those skilled in the art, and it is contemplated that variations on the method described above, such as are described by Tian et al. (1994) or Lazareno and Birdsall (1993), may be used.

Microphysiometric assay

Because cellular metabolism is intricately involved in a broad range of cellular events (including receptor activation of multiple messenger pathways), the use of microphysiometric measurements of cell metabolism can in principle provide a generic assay of cellular activity arising from the activation of any receptor regardless of the specifics of the receptor's signaling pathway.

General guidelines for transient receptor expression, cell preparation and microphysiometric recording are described elsewhere (Salon, J.A. and Owicki, J.A., 1996). Typically cells expressing receptors are harvested and seeded at 3 x 10^5 cells per microphysiometer capsule in complete media 24 hours prior to an experiment. The media is replaced with serum free media 16 hours prior to recording to minimize non-specific metabolic

stimulation by assorted and ill-defined serum factors. On the day of the experiment the cell capsules are transferred to the microphysiometer and allowed to equilibrate in recording media (low buffer RPMI 1640, no bicarbonate, no serum (Molecular Devices Corporation, Sunnyvale, CA) containing 0.1% fatty acid free BSA), during which a baseline measurement of basal metabolic activity is established.

A standard recording protocol specifies a 100 µl/min flow rate, with a 2 min total pump cycle which includes a 30 sec flow interruption during which the acidification rate measurement is taken. Ligand challenges involve a 1 min 20 sec exposure to the sample just prior to the first post challenge rate measurement being taken, followed by two additional pump cycles for a total of 5 min 20 sec sample exposure. Typically, drugs in a primary screen are presented to the cells at 10 µM final concentration.

Follow up experiments to examine dose-dependency of active compounds are then done by sequentially challenging the cells with a drug concentration range that exceeds the amount needed to generate responses ranging from threshold to maximal levels. Ligand samples are then washed out and the acidification rates reported are expressed as a percentage increase of the peak response over the baseline rate observed just prior to challenge.

MAP kinase assay

MAP kinase (mitogen activated kinase) may be monitored to evaluate receptor activation. MAP kinase is activated by multiple pathways in the cell. A primary mode of activation involves the ras/raf/MEK/MAP kinase pathway. Growth factor (tyrosine kinase) receptors feed into this pathway via SHC/Grb-2/SOS/ras. Gi coupled receptors are also known to activate ras and subsequently produce an activation of MAP kinase. Receptors that activate phospholipase C (such as Gq/G11-coupled) produce

diacylglycerol (DAG) as a consequence of phosphatidyl inositol hydrolysis. DAG activates protein kinase C which in turn phosphorylates MAP kinase.

MAP kinase activation can be detected by several approaches. One approach is based on an evaluation of the phosphorylation state, either unphosphorylated (inactive) or phosphorylated (active). The phosphorylated protein has a slower mobility in SDS-PAGE and can therefore be compared with the unstimulated protein using Western blotting. Alternatively, antibodies specific for the phosphorylated protein are available (New England Biolabs) which can be used to detect an increase in the phosphorylated kinase. In either method, cells are stimulated with the test compound and then extracted with Laemmli buffer. The soluble fraction is applied to an SDS-PAGE gel and proteins are transferred electrophoretically to nitrocellulose or Immobilon. Immunoreactive bands are detected by standard Western blotting technique. Visible or chemiluminescent signals are recorded on film and may be quantified by densitometry.

Another approach is based on evaluation of the MAP kinase activity via a phosphorylation assay. Cells are stimulated with the test compound and a soluble extract is prepared. The extract is incubated at 30°C for 10 min with gamma-^{32}P-ATP, an ATP regenerating system, and a specific substrate for MAP kinase such as phosphorylated heat and acid stable protein regulated by insulin, or PHAS-I. The reaction is terminated by the addition of H_3PO_4 and samples are transferred to ice. An aliquot is spotted onto Whatman P81 chromatography paper, which retains the phosphorylated protein. The chromatography paper is washed and counted for ^{32}P in a liquid scintillation counter. Alternatively, the cell extract is incubated with gamma-^{32}P-ATP, an ATP regenerating system, and biotinylated myelin basic protein bound by streptavidin to a filter support. The myelin basic

protein is a substrate for activated MAP kinase. The phosphorylation reaction is carried out for 10 min at 30°C. The extract can then by aspirated through the filter, which retains the phosphorylated myelin basic protein. The filter is washed and counted for ^{32}P by liquid scintillation counting.

Cell proliferation assay

Receptor activation of the receptor may lead to a mitogenic or proliferative response which can be monitored via ^{3}H-thymidine uptake. When cultured cells are incubated with ^{3}H-thymidine, the thymidine translocates into the nuclei where it is phosphorylated to thymidine triphosphate. The nucleotide triphosphate is then incorporated into the cellular DNA at a rate that is proportional to the rate of cell growth. Typically, cells are grown in culture for 1-3 days. Cells are forced into quiescence by the removal of serum for 24 hrs. A mitogenic agent is then added to the media. Twenty-four hours later, the cells are incubated with ^{3}H-thymidine at specific activities ranging from 1 to 10 µCi/ml for 2-6 hrs. Harvesting procedures may involve trypsinization and trapping of cells by filtration over GF/C filters with or without a prior incubation in TCA to extract soluble thymidine. The filters are processed with scintillant and counted for ^{3}H by liquid scintillation counting. Alternatively, adherent cells are fixed in MeOH or TCA, washed in water, and solubilized in 0.05% deoxycholate/0.1 N NaOH. The soluble extract is transferred to scintillation vials and counted for ^{3}H by liquid scintillation counting.

Alternatively, cell proliferation can be assayed by measuring the expression of an endogenous or heterologous gene product, expressed by the cell line used to transfect the receptor, which can be detected by methods such as, but not limited to, florescence intensity, enzymatic activity, immunoreactivity, DNA hybridization, polymerase chain reaction, etc.

Promiscuous second messenger assays

It is not possible to predict, a priori and based solely upon the GPCR sequence, which of the cell's many different signaling pathways any given receptor will naturally use. It is possible, however, to coax receptors of different functional classes to signal through a pre-selected pathway through the use of promiscuous G_α subunits. For example, by providing a cell based receptor assay system with an endogenously supplied promiscuous G_α subunit such as $G_{\alpha 15}$ or $G_{\alpha 16}$ or a chimeric G_α subunit such as $G_{\alpha qz}$, a GPCR, which might normally prefer to couple through a specific signaling pathway (e.g., G_s, G_i, G_q, G_o, etc.), can be made to couple through the pathway defined by the promiscuous G_α subunit and upon agonist activation produce the second messenger associated with that subunit's pathway. In the case of $G_{\alpha 15}$, $G_{\alpha 16}$ and/or $G_{\alpha qz}$ this would involve activation of the G_q pathway and production of the second messenger IP_3. Through the use of similar strategies and tools, it is possible to bias receptor signaling through pathways producing other second messengers such as Ca^{++}, cAMP, and K^+ currents, for example (Milligan, 1999).

It follows that the promiscuous interaction of the exogenously supplied G_α subunit with the receptor alleviates the need to carry out a different assay for each possible signaling pathway and increases the chances of detecting a functional signal upon receptor activation.

Methods for *Xenopus* oocytes preparation, mRNA injection and electrophysiological recording

Female *Xenopus laevis* (*Xenopus*-1, Ann Arbor, MI) were anesthetized in 0.2% tricain (3-aminobenzoic acid ethyl ester, Sigma Chemical Corp.) and a portion of ovary was removed using

aseptic technique (Quick and Lester, 1994). Oocytes were defolliculated, using 3 mg/ml collagenase (Worthington Biochemical Corp., Freehold, NJ) in a solution containing 82.5 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$ and 5 mM HEPES, pH 7.5, and injected (Nanoject, Drummond Scientific, Broomall, PA) 24 h later with 50-70 nl of individual mRNAs or mRNA mixtures (see below).

Elevation of intracellular cAMP is monitored in oocytes by expression of the cystic fibrosis transmembrane conductance regulator (CFTR) whose Cl^--selective pore opens in response to phosphorylation by protein kinase A (Riordan, 1993). To prepare RNA transcripts for expression in oocytes, a template was created by PCR using 5′ and 3′ primers derived from the published sequence of the CFTR gene (Riordan, 1993). The 5′ primer included the sequence coding for T7 polymerase so that transcripts could be generated directly from the PCR products without cloning. Oocytes were injected with 10 ng of CFTR mRNA in addition to 10-15 ng mRNA for SNORF33. Electrophysiological recordings were made after a 2-3 day incubation at 18°C.

Dual electrode voltage clamp ("GeneClamp", Axon Instruments Inc., Foster City, CA) was performed using 3 M KCl-filled glass microelectrodes having resistances of 1-3 Mohms. Unless otherwise specified, oocytes were voltage clamped at a holding potential of -80 mV. During recordings, oocytes were bathed in continuously flowing (1-3 ml/min) medium containing 96 mM NaCl, 2 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, and 5 mM HEPES, pH 7.5 (ND96). Drugs were applied either by local perfusion from a 10 μl glass capillary tube fixed at a distance of 0.5 mm from the oocyte, or for calculation of steady-state EC_{50}s of agonists and for all antagonist experiments, by switching from a series of gravity fed perfusion lines. Experiments were carried out at room temperature. All values are expressed as mean ± standard error of the mean.

Methods for recording currents in *Xenopus* oocytes

Oocytes are harvested from *Xenopus laevis* and injected with mRNA transcripts as previously described (Quick and Lester, 1994; Smith et al.,1997). The test receptor of this invention and Gα subunit RNA transcripts are synthesized using the T7 polymerase ("Message Machine," Ambion) from linearized plasmids or PCR products containing the complete coding region of the genes. Oocytes are injected with 10 ng synthetic receptor RNA and incubated for 3-8 days at 17 degrees. Three to eight hours prior to recording, oocytes are injected with 500 pg promiscuous Gα subunits mRNA in order to observe coupling to Ca^{++} activated Cl^- currents. Dual electrode voltage clamp (Axon Instruments Inc.) is performed using 3 M KCl-filled glass microelectrodes having resistances of 1-2 MOhm. Unless otherwise specified, oocytes are voltage clamped at a holding potential of -80 mV. During recordings, oocytes are bathed in continuously flowing (1-3 ml/min) medium containing 96 mM NaCl, 2 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, and 5 mM HEPES, pH 7.5 (ND96). Drugs are applied either by local perfusion from a 10 µl glass capillary tube fixed at a distance of 0.5 mm from the oocyte, or by switching from a series of gravity fed perfusion lines.

Other oocytes may be injected with a mixture of receptor mRNAs and synthetic mRNA encoding the genes for G-protein-activated inward rectifier channels (GIRK1 and GIRK4, U.S. Patent Nos. 5,734,021 and 5,728,535 or GIRK1 and GIRK2) or any other appropriate combinations (see, e.g., Inanobe et al., 1999). Genes encoding G-protein inwardly rectifying K^+ (GIRK) channels 1, 2 and 4 (GIRK1, GIRK2, and GIRK4) may be obtained by PCR using the published sequences (Kubo et al., 1993; Dascal et al., 1993; Krapivinsky et al., 1995 and 1995b) to derive appropriate 5' and 3' primers. Human heart or brain cDNA may be used as template together with appropriate primers.

Heterologous expression of GPCRs in *Xenopus* oocytes has been widely used to determine the identity of signaling pathways activated by agonist stimulation (Gundersen et al., 1983; Takahashi et al., 1987). Activation of the phospholipase C (PLC) pathway is assayed by applying test compound in ND96 solution to oocytes previously injected with mRNA for the mammalian receptor (with or without promiscuous G proteins) and observing inward currents at a holding potential of -80 mV. The appearance of currents that reverse at -25 mV and display other properties of the Ca^{++}-activated Cl^- (chloride) channel is indicative of mammalian receptor-activation of PLC and release of IP3 and intracellular Ca^{++}. Such activity is exhibited by GPCRs that couple to G_q or G_{11}.

Measurement of inwardly rectifying K^+ (potassium) channel (GIRK) activity may be monitored in oocytes that have been co-injected with mRNAs encoding the mammalian receptor plus GIRK subunits. GIRK gene products co-assemble to form a G-protein activated potassium channel known to be activated (i.e., stimulated) by a number of GPCRs that couple to G_i or G_o (Kubo et al., 1993; Dascal et al., 1993). Oocytes expressing the mammalian receptor plus the GIRK subunits are tested for test compound responsivity by measuring K^+ currents in elevated K^+ solution containing 49 mM K^+.

Membrane preparations

Cell membranes expressing the receptor protein of this invention are useful for certain types of assays including but not restricted to ligand binding assays, GTP-g-S binding assays, and others. The specifics of preparing such cell membranes may in some cases be determined by the nature of the ensuing assay but typically involve harvesting whole cells and disrupting the cell pellet by sonication in ice cold buffer (e.g. 20 mM Tris HCl, mM

EDTA, pH 7.4 at 4° C). The resulting crude cell lysate is cleared of cell debris by low speed centrifugation at 200xg for 5 min at 4° C. The cleared supernatant is then centrifuged at 40,000xg for 20 min at 4° C, and the resulting membrane pellet is washed by suspending in ice cold buffer and repeating the high speed centrifugation step. The final washed membrane pellet is resuspended in assay buffer. Protein concentrations are determined by the method of Bradford (1976) using bovine serum albumin as a standard. The membranes may be used immediately or frozen for later use.

Generation of baculovirus

The coding region of DNA encoding the human receptor disclosed herein may be subcloned into pBlueBacIII into existing restriction sites or sites engineered into sequences 5' and 3' to the coding region of the polypeptides. To generate baculovirus, 0.5 μg of viral DNA (BaculoGold) and 3 μg of DNA construct encoding a polypeptide may be co-transfected into 2 x 10^6 *Spodoptera frugiperda* insect Sf9 cells by the calcium phosphate co-precipitation method, as outlined by Pharmingen (in "Baculovirus Expression Vector System: Procedures and Methods Manual"). The cells then are incubated for 5 days at 27°C.

The supernatant of the co-transfection plate may be collected by centrifugation and the recombinant virus plaque purified. The procedure to infect cells with virus, to prepare stocks of virus and to titer the virus stocks are as described in Pharmingen's manual.

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Labeled ligand binding assays

Cells expressing the receptors of this invention may be used to screen for ligands for said receptors, for example, by labeled [^{3}H]-TYR and [^{3}H]-T binding assays. The same assays may be used to identify agonists or antagonists of the receptors that may be employed for a variety of therapeutic purposes.

[^{3}H]-TYR and [^{3}H]-T binding assays were performed essentially as described by Mallard et al.(1992), with minor modifications. Radioligand binding assays were performed by diluting membranes prepared from cells expressing the receptor in 25 mM Gly-Gly buffer (Sigma, pH = 7.4 @ 0°C) containing 5 mM ascorbate and 10 µM pargyline (final protein concentration in assay = 100 - 300 µg/ml). Membranes were then incubated with either [^{3}H]-TYR (American Radiochemicals, specific activity 60 mCi/µmole) or [^{3}H]-T (Amersham, specific activity 98 mCi/µmole) in the presence or absence of competing ligands on ice for 30 min in a total volume of 250 µl in 96 well microtiter plates. The bound ligand was separated from free by filtration through GF/B filters presoaked in 0.5% polyethyleneimine (PEI), using Tomtec (Wallac) vacuum

filtration device. After addition of Ready Safe (Beckman) scintillation fluid, bound radioactivity was quantitated using a Trilux (Wallac) scintillation counter (approximately 20% counting efficiency of bound counts). Data was fit to non-linear curves using GraphPad Prism.

In this manner, agonist or antagonist compounds that bind to the receptor may be identified as they inhibit the binding of the labeled ligand to the membrane protein of cells expressing the said receptor. Non-specific binding was defined as the amount of radioactivity remaining after incubation of membrane protein in the presence of 10 μM of the unlabeled amine corresponding to the radioligand used. In equilibrium saturation binding assays, membrane preparations or intact cells transfected with the receptor are incubated in the presence of increasing concentrations of the labeled compound to determine the binding affinity of the labeled ligand. The binding affinities of unlabeled compounds may be determined in equilibrium competition binding assays, using a fixed concentration of labeled compound in the presence of varying concentrations of the displacing ligands.

Localization of mRNA coding for human and rat SNORF33.

Quantitative RT-PCR using a fluorogenic probe with real time detection: Quantitative RT-PCR using fluorogenic probes and a panel of mRNA extracted from human and rat tissue was used to characterize the localization of SNORF33 rat and human RNA. This assay utilizes two oligonucleotides for conventional PCR amplification and a third specific oligonucleotide probe that is labeled with a reporter at the 5' end and a quencher at the 3' end of the oligonucleotide. In the instant invention, FAM (6-carboxyfluorescein) and JOE (6 carboxy-4,5-dichloro-2,7-dimethoxyfluorescein) were the two reporters that were utilized

and TAMRA (6-carboxy-4,7,2,7'-tetramethylrhodamine) was the quencher. As amplification progresses, the labeled oligonucleotide probe hybridizes to the gene sequence between the two oligonucleotides used for amplification. The nuclease activity of Taq, or rTth thermostable DNA polymerases is utilized to cleave the labeled probe. This separates the quencher from the reporter and generates a fluorescent signal that is directly proportional to the amount of amplicon generated. This labeled probe confers a high degree of specificity. Non-specific amplification is not detected as the labeled probe does not hybridize. All experiments were conducted in a PE7700 Sequence Detection System (Perkin Elmer, Foster City CA).

Quantitative RT-PCR: For the detection of RNA encoding SNORF33, quantitative RT-PCR was performed on mRNA extracted from tissue. Reverse transcription and PCR reactions were carried out in 50 μl volumes using rTth DNA polymerase (Perkin Elmer). Primers with the following sequences were used:

SNORF33 human:

Forward primer:
SNORF33h 41F
5'-CATGGCCACTGTGGACTTTCT-3' (SEQ ID NO: 22)

Reverse primer
SNORF33h 158R
5'-GTCGGTGCTTGTGTGAATTTTACA-3' (SEQ ID NO: 23)

Fluorogenic oligonucleotide probe:
SNORF33h-90T
5'(6-FAM)-ATGGTGAGATCTGCTGAGCACTGTTGGTATT-(*TAMRA)3'* ((SEQ ID NO: 24)

SNORF33 rat

forward primer
SNORF33R-1067F
5'-TGCATGGTCCTGGACCCT-3' (SEQ ID NO: 25)

reverse primer
SNORF33R.seq-1163R
5'-TCGGGTTGAAGGCAGAGTTC-3' (SEQ ID NO: 26)

Fluorogenic oligonucleotide probe:
SNORF33R-1089T
5'(6-FAM)-TGGGCTATGTTATCCCACCCACTCTGAAT-*(TAMRA)3'* (SEQ ID No: 27)

Using these primer pairs, amplicon length is 117 bp for human SNORF33 and 96 bp for rat SNORF33. Each RT-PCR reaction contained 50 ng mRNA. Oligonuceotide concentrations were: 500 nM of forward and reverse primers, and 200 nM of fluorogenic probe. Concentrations of reagents in each reaction were: 300 μM each of dGTP; dATP; dCTP; 600 μM UTP; 3.0mM Mn(OAc)2 ; 50 mM Bicine; 115 mM potassium acetate, 8% glycerol, 5 units rTth DNA polymerase, and 0.5 units of uracil N-glycosylase. Buffer for RT-PCR reactions also contained a fluor used as a passive reference (ROX: Perkin Elmer proprietary passive reference I). All reagents for RT-PCR (except mRNA and oligonucleotide primers) were obtained from Perkin Elmer (Foster City, CA). Reactions were carried using the following thermal cycler profile: 50°C 2 min., 60°C 30 min., 95°C 5 min., followed by 40 cycles of: 94°C 20 sec., 62°C 1 min.

Positive controls for PCR reactions consisted of amplification of the target sequence from a plasmid construct. Standard curves for quantitation of human and rat SNORF33 were constructed using human genomic DNA or rat stomach RNA. Negative controls

consisted of mRNA blanks, as well as primer and mRNA blanks. To confirm that the mRNA was not contaminated with genomic DNA, PCR reactions were carried out without reverse transcription using Taq DNA polymerase. Integrity of RNA was assessed by amplification of mRNA coding for cyclophilin or glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Following reverse transcription and PCR amplification, data was analyzed using Perkin Elmer sequence detection software. The fluorescent signal from each well was normalized using an internal passive reference, and data was fitted a standard curve to obtain relative quantities of SNORF33 mRNA expression.

In Situ Hybridization experiments for SNORF33 mRNA

Tissue Preparation for neuroanatomical studies: Male Sprague-Dawley rats (Charles Rivers, Rochester, NY) were euthanized using CO_2, decapitated, and their brains and select peripheral tissues immediately removed and rapidly frozen on crushed dry ice. Coronal sections of brain tissue and peripheral tissues were cut at 11 μm using a cryostat and thaw-mounted onto poly-L-lysine-coated slides and stored at -20°C until use. Prior to hybridization, the tissues were fixed in 4% paraformaldehyde/PBS pH 7.4 followed by two washes in PBS (Specialty Media, Lavallette, NJ). Tissues were then treated in 5 mM dithiothreitol, rinsed in DEPC-treated water, acetylated in 0.1 M triethanolamine containing 0.25% acetic anhydride, rinsed twice in 2 x SSC, delipidated with chloroform then dehydrated through a series of graded alcohols. All reagents were purchased from Sigma (St. Louis, MO).

In Situ Hybridization Histochemistry

Oligonucleotide probes, BB1009/1010, corresponding to nucleotides 115-159 of the rat SNORF33 cDNA, were used to characterize the distribution of each receptor's respective mRNA. The oligonucleotides were synthesized using an Expedite Nucleic Acid

Synthesis System (PerSeptive Biosystems, Framingham, MA) and purified using 12% polyacrylamide gel electrophoresis.

The sequences of the rat SNORF33 oligonucleotides are:

Sense probe: BB1009: 5'- CAC ACG AAC AGC AAC TGG TCA AGG GAT GTC CGT GCT TCG CTG TAC-3' (SEQ ID NO: 28)

Antisense probe: B1010: 5'- GTA CAG CGA AGC ACG GAC ATC CCT TGA CCA GTT GCT GTT CGT GTG-3' (SEQ ID No: 29)

Probes were 3'-end labeled with [^{35}S]dATP (1200Ci/mmol, NEN, Boston, MA) to a specific activity of 10^9 dpm/μg using terminal deoxynucleotidyl transferase (Pharmacia, Piscataway, NJ). *In situ* hybridization was done with modification of the method described by Durkin, et al. (1995).

RESULTS AND DISCUSSION

Isolation of a full-length rat SNORF33 receptor

100 ng rat genomic DNA was subjected to MOPAC PCR with two degenerate primers designed based on the sixth and seventh transmembrane domains of select serotonin receptors. One product from this reaction, 5-HT-38-rgen-051, was found to be a novel DNA sequence not found in the Genbank databases (Genembl, STS, EST, GSS), which had 42-48% amino acid identity to $5HT_4$, dopamine D_2 and β-adrenergic receptors. This novel sequence was designated SNORF33.

The full-length rat SNORF33 sequence was acquired by screening a rat genomic phage library with a SNORF33 specific oligonucleotide probe. Southern blot analysis of a single isolated plaque identified a 5.5 kb fragment which was subcloned (KO26) and sequenced. Sequencing of KO26 revealed an open reading frame of 996 bp that is predicted to encode a protein of 332 amino acids. A 1.8 kb fragment of KO26, including the 996 bp open reading frame, was subcloned into pcDNA3.1, resulting in the construct named BO111. The nucleotide sequence of rat SNORF33 and its translated amino acid sequence are represented in Figures 3 and 4, respectively.

Hydophobicity (Kyte-Doolittle) analysis of the amino acid sequence of the full-length clone indicates the presence of seven hydrophobic regions, which is consistent with the seven transmembrane domains of a G protein-coupled receptor. The seven expected transmembrane domains are indicated in Figure 4. A comparison of nucleotide and peptide sequences of rat SNORF33 with sequences contained in the Genbank, EMBL and SwissProtPlus databases reveals that the amino acid sequence of this clone is most related to the $5HT_4$-like pseudogene (44% amino acid

identity) and PNR (GenBank accession number 2465432; 38% amino acid identity), $5HT_{1D}$, $5HT_4$ and dopamine D_1 receptors (35-36% amino acid identities) and histamine H_1 and α_{1c} adrenergic receptors (33% amino acid identity). There were no sequences in the Genbank databases (Genembl, STS, EST, GSS, or SwissProt) that were identical to SNORF33.

SNORF33 has one potential protein kinase C (PKC) phosphorylation motif at serine 325 in the carboxy-terminal tail. It also has three potential N-linked glycosylation sites at asparagines 9 and 14 in the amino-terminal tail and at asparagine 283 in the seventh transmembrane domain.

Isolation of the Human SNORF33 Homologue

A fragment of the human homologue of SNORF33 was amplified from human genomic DNA by low stringency PCR using oligonucleotide primers designed against the rat SNORF33. The sequence of this fragment was then used to generate human SNORF33 PCR primers which were used to amplify under high stringency a SNORF33 fragment from human genomic DNA. This fragment, KO28, contains 573 nucleotides of human SNORF33, from TMII to the beginning of TMVI. The nucleotide and amino acid sequences of the human SNORF33 fragment are shown in Figures 1 and 2, respectively. The human SNORF33 fragment shares 79% nucleotide and amino acid identity with the rat SNORF33.

Isolation of the full-length human SNORF33

To obtain the full-length human SNORF33, 5' and 3' RACE was performed on human kidney and stomach cDNA. The 5' RACE reaction yielded a 500 bp band that contained sequence information through the first transmembrane domain and a putative in-frame initiating methionine-coding sequence. The 3' RACE reaction yielded a 350 bp band that contained sequence for an in-frame stop codon

downstream from the region coding for the seventh transmembrane domain. Two primers, BB1101 and BB1102, were used to amplify the entire coding sequence from human genomic DNA and human amygdala cDNA using the Expand Long Template PCR system. The primers for this reaction were specific to the 5′ and 3′ untranslated regions of SNORF33 with *BamHI* and *Hind*III restriction sites incorporated into the 5′ ends of the 5′ and 3′ primers, respectively. The products of these reactions were subcloned into pcDNA3.1 and sequenced. The full-length human SNORF33 in pcDNA3.1, BO113, was named pcDNA3.1-hSNORF33-f. A *BamHI*/*Hind*III fragment of BO113, containing the entire SNORF33 insert, was ligated into a *BamHI*/*Hind*III digested pEXJ.RHT3T7 vector. This construct, BO114, was named pEXJ-hSNORF33-f. The largest open reading frame in human SNORF33 is 1017 nucleotides (Figures 5A-5B), and predicts a protein a protein of 339 amino acids (Figures 6A-6B). A comparison of the rat and human SNORF33 sequences reveals 79% nucleotide identity and 78% amino acid identity (Figures 7A-7B).

Hydophobicity (Kyte-Doolittle) analysis of the amino acid sequence of the full-length clone indicates the presence of seven hydrophobic regions, which is consistent with the seven transmembrane domains of a G protein-coupled receptor (Figures 6A-6B).

A comparison of nucleotide and peptide sequences of human SNORF33 with sequences contained in the Genbank, EMBL, and SwissProtPlus databases reveals that the amino acid sequence of this clone is most related to the $5HT_4$-like pseudogene (46% amino acid identity) and PNR (GenBank accession number 2465432; 40% amino acid identity), $5HT1_D$ and 5HT4 receptors (35-38% amino acid identities) and histamine H1, dopamine D1 and α1c adrenergic receptors (33-34% amino acid identities). There were no sequences in the Genbank databases (Genembl, STS, EST, GSS, or SwissProt) that were identical to SNORF33.

Human SNORF33 has one potential protein kinase C (PKC) phosphorylation motif at serine 328 in the carboxy-terminal tail. It also has three potential N-linked glycosylation sites at asparagines 10 and 17 in the amino-terminal tail and at asparagine 296 in the seventh transmembrane domain.

Isolation of the Mouse SNORF33 Homologue

A fragment of the mouse homologue of SNORF33 was amplified from mouse genomic DNA by low stringency PCR using oligonucleotide primers designed against the rat SNORF33. The sequence of this fragment was then used to generate mouse SNORF33 PCR primers which were used to amplify under high stringency a SNORF33 fragment from mouse genomic DNA. This fragment, KO94, contains 252 nucleotides of mouse SNORF33, from TMV to the beginning of the third extracellular loop. The amino acid and nucleotide sequences of the mouse SNORF33 fragment are shown in Figures 17 and 18. The mouse SNORF33 fragment shares 93% nucleotide and 92% amino acid identity with the rat SNORF33 receptor. The mouse SNORF33 fragment shares 78% nucleotide and 72% amino acid identity with the human SNORF33 receptor.

Increase in Intracellular cAMP Levels:

COS-7 cells were transiently transfected with rSNORF33 and vector DNA (mock) as described in Materials and Methods. Activation of rSNORF33 receptor by various ligands resulted in enhancement of intracellular cAMP levels (Figure 9, Figure 10, Table 1) but not intracellular Ca^{++} release (measured by FLIPR). In contrast, these ligands had no or significantly less effect on cAMP levels in vector-transfected cells (Figure 9, Figure 10). Interestingly, the basal cAMP levels of rSNORF33-transfected cells were significantly higher than mock-transfected cells (Figure 8). These results suggest that rSNORF33 receptor is positively coupled to adenylyl cyclase, most probably via Gs-class of G-proteins.

Several "trace amines" displayed high potencies of approximately 10-20 nM for stimulating cAMP levels in rSNORF33-transfected cells with the following rank order of potencies TYR> PEA> T. Another "trace amine", OCT, displayed about an order of magnitude lower potency as compared to the above amines (Table 1).

Amphetamine which belongs to the structural class of phenylethylamines showed relatively high potency in the cAMP assay (see Table 1), in fact, it is only about 2-fold less potent than TYR and PEA. Amphetamines are well known for their CNS stimulating and appetite suppressing properties and they are the most potent anorectic compounds known in man as well as in other species. It is noteworthy that the (R)-enantiomer of amphetamine is more active than the (S)- at the rSNORF33, whereas the reverse is true for in vivo CNS stimulating effects of amphetamine on locomotion and neurotransmitter release.

Tryptamine has been shown to have relatively high affinity (1-100 nM Ki values) at several cloned 5-HT receptors. 5-Hydroxytryptamine was found to be 70-fold less potent than the three "trace amines" mentioned above, in activating rSNORF33 (Table 1). Furthermore, TYR and OCT, which have low affinity for 5-HT receptors, activated rSNORF33 with relatively high potencies (Table 1). Similarly, rSNORF33 does not display adrenergic, dopaminergic or histaminergic pharmacology since DA was a much weaker agonist than the three most potent "trace amines" (Table 1) and NE and histamine were inactive at the cloned rSNORF33 receptor (data not shown). Indeed, rSNORF33 displays a unique pharmacological profile unlike any other cloned or native aminergic receptor described previously.

Unexpectedly, several compounds, notably desipramine and fluoxetine, which produce their physiological effects indirectly by inhibiting uptake of neurotransmitters, acted as direct

agonists at rSNORF33 (Table 1). Desipramine and fluoxetine are very effective antidepressants clinically. Since the above mentioned drugs activate rSNORF33, it is possible that some of their physiological effects described are mediated via their actions at rSNORF33.

TABLE 1. Agonist Potencies For Stimulation Of rSNORF33 Receptors As Measured By Intracellular cAMP Release In SNORF33-Transfected COS-7 Cells.

Compounds	Mean* EC_{50} ± S.E.M.# (nM)
Tyramine (TYR)	9 ± 6
Tryptamine (T)	17 ± 4
β-Phenylethylamine (PEA)	10 ± 1
(R)-Amphetamine	17 ± 7
(S)-Amphetamine	43 ± 11
Kynuramine	90 ± 20
Methamphetamine	115 ± 66
Octopamine (OCT)	135 ± 54
5-Fluoro-Tryptamine	232 ± 38
Dopamine (DA)	273 ± 22
5-Methoxy-Tryptamine	414 ± 161
5-Methyl-Tryptamine	752 ± 285
Serotonin (5-HT)	1240 ± 526
Phenylpropanolamine (PPA)	1798 ± 1376
Desipramine	4300 ± 1868
Fluoxetine	5521 ± 3521

* calculated using results from 3-8 experiments

\# S.E.M.; Standard Error of Mean

Activation of currents in SNORF33 expressing *Xenopus* oocytes

The activity of rSNORF33 was tested in oocytes co-injected with mRNA encoding rSNORF33 and mRNA encoding CFTR. Initially, a broad panel of candidate agonists were tested. From this broad panel, OCT and, more weakly, DA and 5-HT, elicited Cl^- currents at 100 μM. Subsequently, several other biogenic amines, including TYR and T(1-100 μM), also produced this activity. These responses were specific to the expression of rSNORF33 since no such currents were observed in other oocytes injected with only mRNA encoding the CFTR channel. Similar currents were observed in oocytes challenged with DA and expressing the dopamine D_1 receptor, which is known to stimulate adenylyl cyclase. The "trace amines" did not stimulate Cl^- currents in oocytes lacking CFTR, indicating that the $G_{\alpha q}$-mediated phospholipase C pathway was not activated. Responses also were not evoked in ooctyes expressing chimeric G-proteins which are able to couple $G_{\alpha i}$-and $G_{\alpha o}$-coupled GPCRs to the phosphclipase C pathway. Taken together, these observations support the conclusion that rSNORF33 encodes a GPCR which binds several trace biogenic amines and stimulates the production of cAMP, presumably via activation of $G_{\alpha s}$.

Quantitative pharmacology was performed on the selected agonists TYR, T, OCT and 5-HT. The effect of stepwise increasing the concentration of agonist on the amplitude of Cl^- current is shown in Figure 11A. Averaged concentration-effect data for selected agonists are shown in Figure 11B. Calculated EC_{50} values for the four agonists were 37 ± 4.4 nM for TYR, 54 ± 10 nM for T, 635 ± 151 nM for OCT and 3776 ± 317 nM for 5-HT. This rank order of potencies is consistent with those obtained for the cAMP responses mediated by rSNORF33 in COS-7 cells and provides support for rSNORF33 being a TYR receptor.

A series of compounds, which included ligands for several biogenic amine receptors, were tested for their ability to antagonize responses elicited by 100 nM TYR (Figure 12). At the test concentration (100 μM except where noted), most of the compounds had little or no significant antagonist activity (< 50% inhibition).

Phenoxybenzamine, which irreversibly blocks several biogenic amine receptors, including T receptors in the rat stomach (Winter and Gessner, 1968), also produced an irreversible inhibition of rSNORF33. Thus, rSNORF33 shares several of the pharmacological properties of T receptors found in the rat brain and periphery.

Antagonists effective at invertebrate OCT and/or TYR receptors, such as mianserin, yohimbine and rauwolscine, did not significantly inhibit TYR stimulation of rSNORF33. This result correlates with the observation that two other compounds that act as antagonists at OCT receptors, phentolamine and cyproheptadine, actually produced an agonist effect. Thus, rSNORF33 is pharmacologically distinct from invertebrate OCT and TYR receptors.

The most potent inhibition was affected by the alpha-1 adrenergic agonist, cirazoline, which produced a greater than 90% inhibition of the response to TYR. It is noteworthy that cirazoline also has additional high affinity for imidazoline receptors. Test compounds having the ability to significantly block the activity of rSNORF33 also included two β-adrenoceptor antagonists, propanolol and pindolol, and the 5-HT receptor antagonist, metergoline. Thus, rSNORF33 shares some of the pharmacological properties of adrenergic, imidazoline and serotonergic receptors.

Human SNORF33 mRNA was transcribed and injected into *Xenopus* oocytes. Three days later, under voltage clamp, currents were

measured in response to the application of 100 μM tyramine (Figure 16). These currents were dependent upon co-expression of the CFTR ion channel, suggesting that they were caused by a receptor mediated elevation of cAMP. Thus, the human homolog, hSNORF33, is a functional receptor strongly stimulated by tyramine.

Receptor Binding

Receptor binding was performed on rSNORF33- and mock-transfected COS-7 membranes using [^{3}H]-TYR and [^{3}H]-T as a radioligand described in the Materials and Methods.

Binding of [^{3}H]-TYR to the rSNORF33 membranes was saturable (Figure 13) and of high affinity (Kd = 12.5 and 14.8 nM, Bmax = 1400 and 1164 fmol/mg protein, n=2). No specific binding sites were present in the mock-transfected membranes. Displacement of [^{3}H]-TYR binding allowed the estimation of binding affinity of a number of compounds for rSNORF33 (Figure 14 and Table 2). The Ki values obtained for compounds displacing [^{3}H]-TYR binding were in good agreement with the potency values obtained for the compounds in the cAMP assay. The trace amines displaced [^{3}H]-TYR binding with a rank order similar to that observed in the functional assays, TYR > β-PEA > T > OCT. In agreement with the results of the functional assay, the (R)-enantiomer of amphetamine demonstrated greater affinity for displacing binding of [^{3}H]-TYR than the (S)-enantiomer.

[^{3}H]-T binding was also explored at the cloned rSNORF33. At 20 nM radioligand concentration, [^{3}H]-T displayed much poorer binding signal (35% specific binding, Figure 15) as compared with [^{3}H]-TYR (90% specific binding at the same concentration, Figure 13) on rSNORF33. This is consistent with both the weaker potency and affinity of T relative to TYR in functional (Table 1) and competition binding studies (Table 2), respectively.

In summary, the pharmacological profile of rSNORF33 described here using functional assays (cAMP release and the oocyte electrophysiological assay), shares several of the pharmacological properties of the TYR and T binding sites described in the literature, namely, relatively high affinity for TYR, PEA, T and kynuramine and low affinity for other classical neurotransmitters such as 5-HT, NE, DA and histamine. However, it is difficult to directly correlate the pharmacological profile of rSNORF33 with that obtained in the literature for the rat, since the cloning of a rat or mammalian TYR receptor has not been published as yet. Furthermore, the described TYR and T receptor pharmacolgy observed in the native systems may not be that of a single TYR or T receptor subtype but may comprise those of several subtypes, whereas drug responses mediated by rSNORF33 shown here are via a single cloned receptor transfected in a heterologous system devoid of any other endogenous TYR and T receptor responses. Moreover, correlation with the cloned TYR or OCT receptor from invertebrates may also be misleading since species differences in amino acid sequence may result in significant differences in the pharmacological profile (e.g. the pharmacological profile of the cloned Drosophila 5-HT receptor, 5-HT_{Dro1} (Witz et al., 1990) does not correlate with any of the known cloned or native mammalian 5-HT receptors). As more information becomes available the relationship between the cloned rat TYR receptor and native "trace amine" binding sites will be clarified.

Since both in functional and binding assays TYR shows the highest potency and affinity, respectively, among the trace amines studied, rSNORF33 is therefore being classified as a TYR receptor.

TABLE 2. Affinities for displacement of $[^3H]$-tyramine at rSNORF33 in transfected COS-7 membranes

Compound	Mean* Ki + S.D.M.# (nM)
tyramine	13 ± 4
tryptamine	70 ± 14
β-phenylethylamine	56 ± 21
(R)-amphetamine	48 ± 28
(S)-amphetamine	226 ± 56
5-Methoxy-Tryptamine	209 ± 68
kynuramine	485 ± 9
methamphetamine	391 ± 246
octopamine	310 ± 71
dopamine	1154 ± 190
serotonin (5-HT)	976 ± 120

* calculated using results from 2-4 experiments

S.D.M.; Standard Deviation of Mean

Detection of mRNA coding for human SNORF33

mRNA was isolated from multiple tissues (listed in Table 3) and assayed as described. Quantitative RT-PCR using a fluorgenic probe demonstrated expression of mRNA encoding human SNORF33 in most tissues assayed (Table 3). Highest levels of human SNORF33 mRNA are found in the kidney, stomach, fetal kidney, small intestine, and fetal lung. Most nervous system structures showed little expression of SNORF33 mRNA as compared to peripheral organs. The notable exception to this is the level of SNORF33 mRNA detected in the amygdala, where mRNA levels are 19% of those detected in the highest expressing tissue, the kidney. Other regions of the human CNS expressing lower levels of SNORF33 mRNA include the hippocampus, the substantia nigra as well as other regions listed in Table 3.

The high levels of human SNORF33 RNA expressed in kidney implicate it in electrolyte regulation and potentially hypertension. It is not known at this time at what site(s) in the kidney this receptor exerts its effects.

Other organs with high levels of SNORF33 mRNA are stomach, and small intestine. The localization to these structures is consistent with functions relating to gastrointestinal motility or absorption. It is not known at this time if human SNORF33 mRNA is localized to ganglion cells, smooth muscle or to mucosal/submucosal layers. Although detected in low levels, the presence of SNORF33 mRNA in multiple regions of the CNS including the amygdala (where levels are highest in the CNS) imply a role in modulating fear, phobias and depression. Its presence in other functionally diverse areas, implies a diffuse regulatory function or regional functionality for this receptor.

Human SNORF33 mRNA appears to be developmentally regulated in the lung. There is an 18-fold decrease in mRNA encoding human SNORF33 in adult lung as compared to fetal tissue. This

implicates human SNORF33 as a potential factor involved in the growth and/or maturation of lungs. The time course of this increase has not been examined and would be important in understanding the function of this receptor.

In summary, the distribution of human SNORF33 mRNA implies renal and gastrointestinal regulatory functions. Its presence in the amygdala suggests modulatory function involving depression and mood disorders. Other CNS structures, although containing low levels of SNORF33 mRNA imply a broad regulatory function in the CNS.

Detection of mRNA coding for rat SNORF33

The tissue showing the highest levels of SNORF33 mRNA is the testes (Table 4). Levels in the testes are more than ten fold higher than any other tissue (see Table 4). This strongly suggests a role in endocrine regulation or reproductive function.

Dorsal root ganglia are the second highest expressing tissues, expressing 8% of the amount found in the testes. The thalamus, spinal cord and the medulla contain lower levels of SNORF33, however, they are the highest levels detected in the CNS. The presence of SNORF33 mRNA in primary sensory neurons, and these CNS regions suggests a modulatory role in pain or sensory transmission. Additionally, it may play a role in modulating autonomic centers present in the medulla.

Rat SNORF33 mRNA is also detected in the gastrointestinal tract. It is detected in the stomach, duodenum, and colon. As in the human, the localization to these structures is consistent with functions relating to gastrointestinal motility or absorption. Detailed localization using *in situ* hybridization in the stomach have been completed and a description follows. Other areas assayed expressing SNORF33 RNA include adipose tissue, kidney, urinary bladder, liver, lung, pancreas and other areas (see Table

4).

Adipose tissue is the third highest expressing tissue in the periphery (testes and stomach being the two highest expressing in the periphery), showing 2% of the amount found in the testes.

In summary, the localization of high levels of SNORF33 to the rat testes suggests a role in reproductive function or endocrine regulation. The high levels present in the dorsal root ganglia, along with detectable levels in the spinal cord, thalamus and medulla strongly suggest a role in sensory transmission. As in the human, there is a suggestion of renal and gastrointestinal regulatory functions. The presence of SNORF33 mRNA in adipose tissue and its coupling to stimulation of cAMP, suggests that this receptor may increase lipolysis, fat mobilization and metabolism, resulting in reduction in body weight, analogous to the action of other cAMP-stimulatory receptors (e.g. β_3-adrenergic) on this tissue.

Other peripheral organs and CNS structures, although containing low levels of SNORF33, mRNA imply a broad regulatory role for this receptor.

Table 3

Distribution of mRNA coding for human SNORF33 receptors using qRT-PCR

mRNA encoding human SNORF33 is expressed as % of highest expressing tissue, kidney.

Region	qRT-PCR % of max	Potential applications
heart	1.19	Cardiovascular indications
kidney	100	Hypertension, electrolyte balance
liver	9.71	Diabetes
lung	2.45	Respiratory disorders, asthma
pancreas	1.34	Diabetes, endocrine disorders
pituitary	2.04	Endocrine/neuroendocrine regulation
placenta	0.44	Gestational abnormalities
small intestine	44.22	Gastrointestinal disorders
spleen	1.98	Immune disorders
stomach	88.02	Gastrointestinal disorders
striated muscle	4.3	Musculoskeletal disorders
amygdala	19.18	Depression, phobias, anxiety, mood disorders
caudate-putamen	0.55	Modulation of dopaminergic function
cerebellum	2.04	Motor coordination
hippocampus	3.28	Cognition/memory
hypothalamus	not detected	Appetite/obesity, neuroendocrine regulation,
spinal cord	1.05	Analgesia, Sensory Modulation and Transmission, Modulation of Autonomic Function
substantia nigra	3.06	Modulation of dopaminergic function. Modulation of motor coordination.
thalamus	Not detected	Sensory integration disorders
whole brain	0.41	
fetal brain	1.34	Developmental disorders
fetal lung	42.98	Developmental disorders
fetal kidney	63.64	Developmental disorders
fetal liver	5.12	Developmental disorders

Table 4

Summary of distribution of mRNA coding for rat SNORF33 receptors mRNA encoding rat SNORF33 is expressed as % of highest expressing tissue (testes).

Tissue	qRT-PCR % of max	Potential applications
adipose	2.05	metabolic disorders
adrenal cortex	not detected	regulation of steroid hormones
adrenal medulla	not detected	regulation of epinephrine release
amygdala	0.05	depression, phobias, anxiety, mood disorders
aorta	0.07	cardiovascular indications
celiac plexus	0.49	modulation of autonomic function
cerebellum	trace	motor coordination
cerebral cortex	not detected	Sensory and motor integration, cognition
choroid plexus	trace	regulation of cerebrospinal fluid
colon	0.91	gastrointestinal disorders
dorsal root ganglia	7.58	sensory transmission
duodenum	1.80	gastrointestinal disorders
heart	0.06	cardiovascular indications
hippocampus	not detected	cognition/memory
hypothalamus	0.10	appetite/obesity, neuroendocrine regulation
kidney	0.05	electrolyte balance, hypertension
liver	0.32	diabetes
lung	0.21	respiratory disorders, asthma
medulla	0.71	analgesia, modulation of autonomic function, sensory transmission and modulation
nucleus accumbens	not detected	regulation of dopaminergic function, drug addiction, neuropsychiatric disorders
olfactory bulb	0.05	olfaction
ovary	Trace	reproductive function
pancreas	0.09	diabetes, endocrine disorders
pineal	trace	regulation of melatonin release
pituitary	not detected	endocrine/neuroendocrine regulation
retina	0.10	visual disorders
spinal cord	0.27	analgesia, sensory modulation and transmission

Tissue	qRT-PCR % of max	Potential applications
spleen	0.05	immune disorders
stomach	6.92	gastrointestinal disorders
striatum	not detected	modulation of dopaminergic function, motor disorders
substantia nigra	not detected	modulation of dopaminergic function, modulation of motor coordination
testes	100	reproductive function
thalamus	5.80	sensory integration disorders
thymus	0.23	immune disorders
trigeminal ganglia	not detected	sensory transmission
urinary bladder	0.76	urinary incontinence
uterus	not detected	reproductive disorders
vas deferens	0.27	reproductive function

In Situ Hybridization experiments for SNORF33 mRNA

The expression of SNORF33 mRNA was examined in a variety of selected rat peripheral tissues, namely, lung, stomach, spleen, liver, kidney, and testes. The kidney and testes were devoid of any hybridization signal for SNORF33 mRNA.

Throughout the body of the stomach a moderate hybridization signal for SNORF33 mRNA was detected over enteric ganglion cells within the muscularis layer located between the outer longitudinal and inner circular layers. A moderate signal was also observed to be related to the cells lining the lumen of the stomach.

$[^3H]$-T binding sites have been reported to be present in the stomach fundus (Brüning and Rommelspacher, 1984). Several 5-HT receptor subtypes have been pharmacologically identified in the rat enteric ganglia, and/or stomach fundus, namely 5-HT_{2B}, 5-HT_3, 5-HT_4 and 5-HT_{1F} and all of these except 5-HT_{1P}, have been cloned. The pharmacological profile of SNORF33 does not match the profile of any of these receptors (Table 1, Figure 12 and Boess and Martin, 1994). These data support the existence of multiple

neuronal target sites for "trace amines" in the stomach fundus and suggest a 5-HT-independent effect for "trace amines" on stomach.

In the spleen, cells positive for SNORF33 mRNA were observed to be located primarily in the red pulp and around the marginal zone of the white pulp. Silver grains were detected over monocytes and scattered eosinophils. Monocytes present in the blood are sequestered in the spleen where they are transformed into macrophages and maintain their phagocytic activity in the spleen. Monocytes that have been removed from the circulation are isolated in the white pulp, the marginal zone and the red pulp. Monocyte/macrophages are active in pinocytosis and phagocytosis. They are involved in the production of antibodies and in cell-mediated immune responses, for example transplant rejections and delayed hypersensitivity reactions. Macrophages are involved in processing and presenting an antigen to lymphocytes thus triggering the proliferation of T- and B-lymphocytes. Eosinophils are motile phagocytotic granulocytes that may also be stored in the spleen. Eosinophils normally constitute 2 to 4% of the circulating white blood cells with a distinctive function in that they kill the larvae of parasites. In the rat, eosinophils are released from the blood to the spleen where they finalize their maturation before they enter the general circulation or can be stored and rapidly delivered to the circulation when needed.

The infusion of T through the pulmonary circulation of isolated lungs of the rat results in the release of a spasmogen resembling slow reacting substance of anaphylaxis and a PGE-like activity. The pharmacology of the release receptor was shown to closely resemble T receptors in the rat stomach strip (Bakhle et al., 1977). The localization of SNORF33 mRNA in the lung appeared to be restricted to monocytes. In the lung's alveolar interstitium there is a resident macrophage population, in addition to

scavenging alveolar macrophages moving through the alveolar fluid along the epithelial surface, which keeps the lung clear of pathogens.

SNORF33 mRNA was identified in scattered monocytes throughout the parenchyma of the liver. The liver is essential for life and it functions as an endocrine and exocrine gland, during certain diseases it is a site of hematopoesis. The liver contains an abundance of phagocytes and is a principal filter for foreign particulate matter, especially bacteria from the alimentary tract.

The identification of SNORF33 mRNA in leucocytes in the above mentioned peripheral tissues suggests a potential role for this receptor as part of the host defense and immune systems of the body.

Overall, the results of the localization studies using *in situ* hybridization and quantitative RT-PCR are in agreement. *In situ* hybridization histochemistry demonstrates SNORF33 (rat) present in enteric ganglion cells as well as mucosal cells in the stomach. Other areas expressing SNORF33 mRNA include immune cells in the spleen, lung, and stomach. Quantitative RT-PCR detected SNORF33 mRNA in these areas, as well as others. The broader distribution of rat SNORF33 mRNA using quantitative RT-PCR reflects higher sensitivity of quantitative RT-PCR, with the concomitant loss of information regarding tissue architecture. Regional expression patterns within a tissue affect visualization of mRNA using *in situ* hybridization. If the SNORF 33 mRNA is distributed diffusely throughout a broad area it is less likely to be detected by *in situ* hybridization. In contrast, if a tissue has low levels of SNORF33 RNA concentrated in a restricted area, *in situ* will be able detect this RNA with a high degree of anatomical precision.

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What is claimed is:

1. An isolated nucleic acid encoding a mammalian SNORF33 receptor.

2. The nucleic acid of claim 1, wherein the nucleic acid is DNA.

3. The DNA of claim 2, wherein the DNA is cDNA.

4. The DNA of claim 2, wherein the DNA is genomic DNA.

5. The nucleic acid of claim 1, wherein the nucleic acid is RNA.

6. The nucleic acid of claim 1, wherein the mammalian SNORF33 receptor is a human SNORF33 receptor.

7. The nucleic acid of claim 6, wherein the human SNORF33 receptor has an amino acid sequence identical to that encoded by the plasmid pcDNA3.1-hSNORF33-f (Patent Deposit Designation PTA-398).

8. The nucleic acid of claim 6, wherein the human SNORF33 receptor has an amino acid sequence identical to that encoded by the plasmid pEXJ-hSNORF33-f (Patent Deposit Designation PTA-570).

9. The nucleic acid of claim 6, wherein the human SNORF33 receptor has an amino acid sequence identical to the amino acid sequence shown in Figures 6A-6B (SEQ ID NO: 6).

10. The nucleic acid of claim 1, wherein the mammalian SNORF33 receptor is a rat SNORF33 receptor.

11. The nucleic acid of claim 9, wherein the rat SNORF33 receptor has an amino acid sequence identical to that encoded by the plasmid pcDNA3.1-rSNORF33-f (Patent Deposit Designation PTA-102).

12. The nucleic acid of claim 9, wherein the rat SNORF33 receptor has an amino acid sequence identical to the amino acid sequence shown in Figures 4A-4B (SEQ ID NO: 4).

13. A purified mammalian SNORF33 receptor protein.

14. The purified mammalian SNORF33 receptor protein of claim 12, wherein the SNORF33 receptor protein is a human SNORF33 receptor protein.

15. The purified mammalian SNORF33 receptor protein of claim 12, wherein the SNORF33 receptor protein is a rat SNORF33 receptor protein.

16. A vector comprising the nucleic acid of claim 1.

17. A vector comprising the nucleic acid of claim 6.

18. A vector of claim 16 or 17 adapted for expression in a cell which comprises the regulatory elements necessary for expression of the nucleic acid in the cell operatively linked to the nucleic acid encoding the receptor so as to permit expression thereof, wherein the cell is a bacterial, amphibian, yeast, insect or mammalian cell.

19. The vector of claim 18, wherein the vector is a baculovirus.

20. The vector of claim 16, wherein the vector is a plasmid.

21. The plasmid of claim 20 designated pcDNA3.1-hSNORF33-f

(Patent Deposit Designation PTA-398).

22. The plasmid of claim 20 designated pEXJ-hSNORF33-f (Patent Deposit Designation PTA-570).

23. The plasmid of claim 20 designated pcDNA3.1-rSNORF33-f (Patent Deposit Designation PTA-102).

24. A cell comprising the vector of claim 18.

25. A cell of claim 24, wherein the cell is a non-mammalian cell.

26. A cell of claim 25, wherein the non-mammalian cell is a *Xenopus* oocyte cell or a *Xenopus* melanophore cell.

27. A cell of claim 24, wherein the cell is a mammalian cell.

28. A mammalian cell of claim 27, wherein the cell is a COS-7 cell, a 293 human embryonic kidney cell, a NIH-3T3 cell, a LM(tk-) cell, a mouse Y1 cell, or a CHO cell.

29. A cell of claim 24, wherein the cell is an insect cell.

30. An insect cell of claim 29, wherein the insect cell is an Sf9 cell, an Sf21 cell or a Trichoplusia ni 5B-4 cell.

31. A membrane preparation isolated from the cell of any one of claims 24, 25, 27, 28, 29 or 30.

32. A nucleic acid probe comprising at least 15 nucleotides, which probe specifically hybridizes with a nucleic acid encoding a mammalian SNORF33 receptor, wherein the probe has a sequence complementary to a unique sequence present within one of the two strands of the nucleic acid encoding

the mammalian SNORF33 receptor contained in plasmid pcDNA3.1-hSNORF33-f (Patent Deposit Designation PTA-398).

33. A nucleic acid probe comprising at least 15 nucleotides, which probe specifically hybridizes with a nucleic acid encoding a mammalian SNORF33 receptor, wherein the probe has a sequence complementary to a unique sequence present within one of the two strands of the nucleic acid encoding the mammalian SNORF33 receptor contained in plasmid pEXJ-hSNORF33-f (Patent Deposit Designation PTA-570).

34. A nucleic acid probe comprising at least 15 nucleotides, which probe specifically hybridizes with a nucleic acid encoding a mammalian SNORF33 receptor, wherein the probe has a sequence complementary to a unique sequence present within one of the two strands of the nucleic acid encoding the mammalian SNORF33 receptor contained in plasmid pcDNA3.1-rSNORF33-f (Patent Deposit Designation PTA-102).

35. A nucleic acid probe comprising at least 15 nucleotides, which probe specifically hybridizes with a nucleic acid encoding a mammalian SNORF33 receptor, wherein the probe has a sequence complementary to a unique sequence present within (a) the nucleic acid sequence shown in Figures 5A-5B (SEQ ID NO: 5) or (b) the reverse complement thereof.

36. A nucleic acid probe comprising at least 15 nucleotides, which probe specifically hybridizes with a nucleic acid encoding a mammalian SNORF33 receptor, wherein the probe has a sequence complementary to a unique sequence present within (a) the nucleic acid sequence shown in Figures 3A-3B (SEQ ID NO: 3) or (b) the reverse complement thereof.

37. The nucleic acid probe of claim 35 or 36, wherein the nucleic acid is DNA.

38. The nucleic acid probe of claim 35 or 36, wherein the nucleic acid is RNA.

39. An antisense oligonucleotide having a sequence capable of specifically hybridizing to the RNA of claim 5, so as to prevent translation of the RNA.

40. An antisense oligonucleotide having a sequence capable of specifically hybridizing to the genomic DNA of claim 4, so as to prevent transcription of the genomic DNA.

41. An antisense oligonucleotide of claim 39 or 40, wherein the oligonucleotide comprises chemically modified nucleotides or nucleotide analogues.

42. An antibody capable of binding to a mammalian SNORF33 receptor encoded by the nucleic acid of claim 1.

43. An antibody of claim 42, wherein the mammalian SNORF33 receptor is a human SNORF33 receptor or a rat SNORF33 receptor.

44. An agent capable of competitively inhibiting the binding of the antibody of claim 42 to a mammalian SNORF33 receptor.

45. An antibody of claim 42, wherein the antibody is a monoclonal antibody or antisera.

46. A pharmaceutical composition comprising (a) an amount of the oligonucleotide of claim 39 capable of passing through a cell membrane and effective to reduce expression of a mammalian SNORF33 receptor and (b) a pharmaceutically acceptable carrier capable of passing through the cell membrane.

47. A pharmaceutical composition of claim 46, wherein the oligonucleotide is coupled to a substance which inactivates mRNA.

48. A pharmaceutical composition of claim 47, wherein the substance which inactivates mRNA is a ribozyme.

49. A pharmaceutical composition of claim 47, wherein the pharmaceutically acceptable carrier comprises a structure which binds to a mammalian SNORF33 receptor on a cell capable of being taken up by the cells after binding to the structure.

50. A pharmaceutical composition of claim 49; wherein the pharmaceutically acceptable carrier is capable of binding to a mammalian SNORF33 receptor which is specific for a selected cell type.

51. A pharmaceutical composition which comprises an amount of the antibody of claim 42 effective to block binding of a ligand to a human SNORF33 receptor and a pharmaceutically acceptable carrier.

52. A transgenic, nonhuman mammal expressing DNA encoding a mammalian SNORF33 receptor of claim 1.

53. A transgenic, nonhuman mammal comprising a homologous recombination knockout of the native mammalian SNORF33 receptor.

54. A transgenic, nonhuman mammal whose genome comprises antisense DNA complementary to the DNA encoding a mammalian SNORF33 receptor of claim 1 so placed within the genome as to be transcribed into antisense mRNA which is

complementary to and hybridizes with mRNA encoding the mammalian SNORF33 receptor so as to thereby reduce translation of such mRNA and expression of such receptor.

55. The transgenic, nonhuman mammal of claim 52 or 53, wherein the DNA encoding the mammalian SNORF33 receptor additionally comprises an inducible promoter.

56. The transgenic, nonhuman mammal of claim 52 or 53, wherein the DNA encoding the mammalian SNORF33 receptor additionally comprises tissue specific regulatory elements.

57. A transgenic, nonhuman mammal of claim 52, 53, or 54, wherein the transgenic, nonhuman mammal is a mouse.

58. A process for identifying a chemical compound which specifically binds to a mammalian SNORF33 receptor which comprises contacting cells containing DNA encoding, and expressing on their cell surface, the mammalian SNORF33 receptor, wherein such cells do not normally express the mammalian SNORF33 receptor, with the compound under conditions suitable for binding, and detecting specific binding of the chemical compound to the mammalian SNORF33 receptor.

59. A process for identifying a chemical compound which specifically binds to a mammalian SNORF33 receptor which comprises contacting a membrane preparation from cells containing DNA encoding, and expressing on their cell surface, the mammalian SNORF33 receptor, wherein such cells do not normally express the mammalian SNORF33 receptor, with the compound under conditions suitable for binding, and detecting specific binding of the chemical compound to the mammalian SNORF33 receptor.

60. The process of claim 58 or 59, wherein the mammalian SNORF33 receptor is a human SNORF33 receptor.

61. The process of claim 58 or 59, wherein the mammalian SNORF33 receptor has substantially the same amino acid sequence as the human SNORF33 receptor encoded by plasmid pcDNA3.1-hSNORF33-f (Patent Deposit Designation PTA-398).

62. The process of claim 58 or 59, wherein the mammalian SNORF33 receptor has substantially the same amino acid sequence as the human SNORF33 receptor encoded by plasmid pEXJ-hSNORF33-f (Patent Deposit Designation PTA-570).

63. The process of claim 58 or 59, wherein the mammalian SNORF33 receptor has substantially the same amino acid sequence as that shown in Figures 6A-6B (SEQ ID NO: 6).

64. The process of claim 58 or 59, wherein the mammalian SNORF33 receptor has the amino acid sequence shown in Figures 6A-6B (SEQ ID NO: 6).

65. The process of claim 58 or 59, wherein the mammalian SNORF33 receptor is a rat SNORF33 receptor.

66. The process of claim 58 or 59, wherein the mammalian SNORF33 receptor has substantially the same amino acid sequence as the rat SNORF33 receptor encoded by plasmid pcDNA3.1-rSNORF33-f (Patent Deposit Designation PTA-102).

67. The process of claim 58 or 59, wherein the mammalian SNORF33 receptor has substantially the same amino acid sequence as that shown in Figures 4A-4B (SEQ ID NO: 4).

68. The process of claim 58 or 59, wherein the mammalian SNORF33 receptor has the amino acid sequence shown in Figures 4A-4B

(SEQ ID NO: 4).

69. The process of claim 58 or 59, wherein the compound is not previously known to bind to a mammalian SNORF33 receptor.

70. A compound identified by the process of claim 69.

71. A process of claim 58 or 59, wherein the cell is an insect cell.

72. The process of claim 58 or 59, wherein the cell is a mammalian cell.

73. The process of claim 72, wherein the cell is nonneuronal in origin.

74. The process of claim 73, wherein the nonneuronal cell is a COS-7 cell, 293 human embryonic kidney cell, a CHO cell, a NIH-3T3 cell, a mouse Y1 cell, or a LM(tk-) cell.

75. A process of claim 72, wherein the compound is a compound not previously known to bind to a mammalian SNORF33 receptor.

76. A compound identified by the process of claim 75.

77. A process involving competitive binding for identifying a chemical compound which specifically binds to a mammalian SNORF33 receptor which comprises separately contacting cells expressing on their cell surface the mammalian SNORF33 receptor, wherein such cells do not normally express the mammalian SNORF33 receptor, with both the chemical compound and a second chemical compound known to bind to the receptor, and with only the second chemical compound, under conditions suitable for binding of such

compounds to the receptor, and detecting specific binding of the chemical compound to the mammalian SNORF33 receptor, a decrease in the binding of the second chemical compound to the mammalian SNORF33 receptor in the presence of the chemical compound being tested indicating that such chemical compound binds to the mammalian SNORF33 receptor.

78. A process involving competitive binding for identifying a chemical compound which specifically binds to a mammalian SNORF33 receptor which comprises separately contacting a membrane preparation from cells expressing on their cell surface the mammalian SNORF33 receptor, wherein such cells do not normally express the mammalian SNORF33 receptor, with both the chemical compound and a second chemical compound known to bind to the receptor, and with only the second chemical compound, under conditions suitable for binding of such compounds to the receptor, and detecting specific binding of the chemical compound to the mammalian SNORF33 receptor, a decrease in the binding of the second chemical compound to the mammalian SNORF33 receptor in the presence of the chemical compound being tested indicating that such chemical compound binds to the mammalian SNORF33 receptor.

79. A process of claim 77 or 78, wherein the mammalian SNORF33 receptor is a human SNORF33 receptor.

80. A process of claim 77 or 78, wherein the mammalian SNORF33 receptor is a rat SNORF33 receptor.

81. The process of claim 77 or 78, wherein the cell is an insect cell.

82. The process of claim 77 or 78, wherein the cell is a mammalian cell.

83. The process of claim 82, wherein the cell is nonneuronal in origin.

84. The process of claim 83, wherein the nonneuronal cell is a COS-7 cell, 293 human embryonic kidney cell, a CHO cell, a NIH-3T3 cell, a mouse Y1 cell, or a LM(tk-) cell.

85. The process of claim 84, wherein the compound is not previously known to bind to a mammalian SNORF33 receptor.

86. A compound identified by the process of claim 85.

87. A method of screening a plurality of chemical compounds not known to bind to a mammalian SNORF33 receptor to identify a compound which specifically binds to the mammalian SNORF33 receptor, which comprises

(a) contacting cells transfected with, and expressing, DNA encoding the mammalian SNORF33 receptor with a compound known to bind specifically to the mammalian SNORF33 receptor;

(b) contacting the cells of step (a) with the plurality of compounds not known to bind specifically to the mammalian SNORF33 receptor, under conditions permitting binding of compounds known to bind to the mammalian SNORF33 receptor;

(c) determining whether the binding of the compound known to bind to the mammalian SNORF33 receptor is reduced in the presence of the plurality of compounds, relative to the binding of the compound in the absence of the plurality of compounds; and if so

(d) separately determining the binding to the mammalian SNORF33 receptor of each compound included in the plurality of compounds, so as to thereby identify any compound included therein which specifically binds to the mammalian SNORF33 receptor.

88. A method of screening a plurality of chemical compounds not known to bind to a mammalian SNORF33 receptor to identify a compound which specifically binds to the mammalian SNORF33 receptor, which comprises

(a) contacting a membrane preparation from cells transfected with, and expressing, DNA encoding the mammalian SNORF33 receptor with the plurality of compounds not known to bind specifically to the mammalian SNORF33 receptor under conditions permitting binding of compounds known to bind to the mammalian SNORF33 receptor;

(b) determining whether the binding of a compound known to bind to the mammalian SNORF33 receptor is reduced in the presence of the plurality of compounds, relative to the binding of the compound in the absence of the plurality of compounds; and if so

(c) separately determining the binding to the mammalian SNORF33 receptor of each compound included in the plurality of compounds, so as to thereby identify any compound included therein which specifically binds to the mammalian SNORF33 receptor.

89. A method of claim 87 or 88, wherein the mammalian SNORF33 receptor is a human SNORF33 receptor.

90. A method of claim 87 or 88, wherein the mammalian SNORF33

receptor is a rat SNORF33 receptor.

91. A method of claim 87 or 88, wherein the cell is a mammalian cell.

92. A method of claim 91, wherein the mammalian cell is non-neuronal in origin.

93. The method of claim 92, wherein the non-neuronal cell is a COS-7 cell, a 293 human embryonic kidney cell, a LM(tk-) cell, a CHO cell, a mouse Y1 cell, or an NIH-3T3 cell.

94. A method of detecting expression of a mammalian SNORF33 receptor by detecting the presence of mRNA coding for the mammalian SNORF33 receptor which comprises obtaining total mRNA from the cell and contacting the mRNA so obtained with the nucleic acid probe of claim 32, 33, 34, 35 or 36 under hybridizing conditions, detecting the presence of mRNA hybridized to the probe, and thereby detecting the expression of the mammalian SNORF33 receptor by the cell.

95. A method of detecting the presence of a mammalian SNORF33 receptor on the surface of a cell which comprises contacting the cell with the antibody of claim 42 under conditions permitting binding of the antibody to the receptor, detecting the presence of the antibody bound to the cell, and thereby detecting the presence of the mammalian SNORF33 receptor on the surface of the cell.

96. A method of determining the physiological effects of varying levels of activity of mammalian SNORF33 receptors which comprises producing a transgenic, nonhuman mammal of claim 52 whose levels of mammalian SNORF33 receptor activity are varied by use of an inducible promoter which regulates mammalian SNORF33 receptor expression.

97. A method of determining the physiological effects of varying levels of activity of mammalian SNORF33 receptors which comprises producing a panel of transgenic, nonhuman mammals of claim 52 each expressing a different amount of mammalian SNORF33 receptor.

98. A method for identifying an antagonist capable of alleviating an abnormality wherein the abnormality is alleviated by decreasing the activity of a mammalian SNORF33 receptor comprising administering a compound to the transgenic, nonhuman mammal of claim 52, 53, or 54, and determining whether the compound alleviates any physiological and/or behavioral abnormality displayed by the transgenic, nonhuman mammal as a result of overactivity of a mammalian SNORF33 receptor, the alleviation of such an abnormality identifying the compound as an antagonist.

99. The method of claim 98, wherein the mammalian SNORF33 receptor is a human SNORF33 receptor or a rat SNORF33 receptor.

100. An antagonist identified by the method of claim 98.

101. A composition comprising an antagonist of claim 100 and a carrier.

102. A method of treating an abnormality in a subject wherein the abnormality is alleviated by decreasing the activity of a mammalian SNORF33 receptor which comprises administering to the subject an effective amount of the pharmaceutical composition of claim 101, thereby treating the abnormality.

103. A method for identifying an agonist capable of alleviating an abnormality in a subject wherein the abnormality is

alleviated by increasing the activity of a mammalian SNORF33 receptor comprising administering a compound to the transgenic, nonhuman mammal of claim 52, 53, or 54, and determining whether the compound alleviates any physiological and/or behavioral abnormality displayed by the transgenic, nonhuman mammal, the alleviation of such an abnormality identifying the compound as an agonist.

104. The method of claim 103, wherein the mammalian SNORF33 receptor is a human SNORF33 receptor or a rat SNORF33 receptor.

105. An agonist identified by the method of claim 104.

106. A composition comprising an agonist identified by the method of claim 105 and a carrier.

107. A method of treating an abnormality in a subject wherein the abnormality is alleviated by increasing the activity of a mammalian SNORF33 receptor which comprises administering to the subject an effective amount of the composition of claim 106 so as to thereby treat the abnormality.

108. A method for diagnosing a predisposition to a disorder associated with the activity of a specific mammalian allele which comprises:

(a) obtaining DNA of subjects suffering from the disorder;

(b) performing a restriction digest of the DNA with a panel of restriction enzymes;

(c) electrophoretically separating the resulting DNA fragments on a sizing gel;

(d) contacting the resulting gel with a nucleic acid probe capable of specifically hybridizing with a unique sequence included within the sequence of a nucleic acid molecule encoding a mammalian SNORF33 receptor and labeled with a detectable marker;

(e) detecting labeled bands which have hybridized to the DNA encoding a mammalian SNORF33 receptor of claim 1 to create a unique band pattern specific to the DNA of subjects suffering from the disorder;

(f) repeating steps (a)-(e) with DNA obtained for diagnosis from subjects not yet suffering from the disorder; and

(g) comparing the unique band pattern specific to the DNA of subjects suffering from the disorder from step (e) with the band pattern from step (f) for subjects not yet suffering from the disorder so as to determine whether the patterns are the same or different and thereby diagnose predisposition to the disorder if the patterns are the same.

109. The method of claim 108, wherein a disorder associated with the activity of a specific mammalian allele is diagnosed.

110. A method of preparing the purified mammalian SNORF33 receptor of claim 13 which comprises:

(a) culturing cells which express the mammalian SNORF33 receptor;

(b) recovering the mammalian SNORF33 receptor from the cells; and

(c) purifying the mammalian SNORF33 receptor so recovered.

111. A method of preparing the purified mammalian SNORF33 receptor of claim 13 which comprises:

(a) inserting a nucleic acid encoding the mammalian SNORF33 receptor into a suitable expression vector;

(b) introducing the resulting vector into a suitable host cell;

(c) placing the resulting host cell in suitable conditions permitting the production of the mammalian SNORF33 receptor;

(d) recovering the mammalian SNORF33 receptor so produced; and optionally

(e) isolating and/or purifying the mammalian SNORF33 receptor so recovered.

112. A process for determining whether a chemical compound is a mammalian SNORF33 receptor agonist which comprises contacting cells transfected with and expressing DNA encoding the mammalian SNORF33 receptor with the compound under conditions permitting the activation of the mammalian SNORF33 receptor, and detecting any increase in mammalian SNORF33 receptor activity, so as to thereby determine whether the compound is a mammalian SNORF33 receptor agonist.

113. A process for determining whether a chemical compound is a mammalian SNORF33 receptor antagonist which comprises contacting cells transfected with and expressing DNA encoding the mammalian SNORF33 receptor with the compound

in the presence of a known mammalian SNORF33 receptor agonist, under conditions permitting the activation of the mammalian SNORF33 receptor, and detecting any decrease in mammalian SNORF33 receptor activity, so as to thereby determine whether the compound is a mammalian SNORF33 receptor antagonist.

114. A process of claim 112 or 113, wherein the mammalian SNORF33 receptor is a human SNORF33 receptor or a rat SNORF33 receptor.

115. A composition which comprises an amount of a mammalian SNORF33 receptor agonist determined by the process of claim 112 effective to increase activity of a mammalian SNORF33 receptor and a carrier.

116. A composition of claim 115, wherein the mammalian SNORF33 receptor agonist is not previously known.

117. A composition which comprises an amount of a mammalian SNORF33 receptor antagonist determined by the process of claim 113 effective to reduce activity of a mammalian SNORF33 receptor and a carrier.

118. A composition of claim 117, wherein the mammalian SNORF33 receptor antagonist is not previously known.

119. A process for determining whether a chemical compound specifically binds to and activates a mammalian SNORF33 receptor, which comprises contacting cells producing a second messenger response and expressing on their cell surface the mammalian SNORF33 receptor, wherein such cells do not normally express the mammalian SNORF33 receptor, with the chemical compound under conditions suitable for activation of the mammalian SNORF33 receptor, and measuring

the second messenger response in the presence and in the absence of the chemical compound, a change in the second messenger response in the presence of the chemical compound indicating that the compound activates the mammalian SNORF33 receptor.

120. The process of claim 119, wherein the second messenger response comprises chloride channel activation and the change in second messenger is an increase in the level of chloride current.

121. The process of claim 119, wherein the second messenger response comprises intracellular calcium levels and the change in second messenger is an increase in the measure of intracellular calcium.

122. The process of claim 119, wherein the second messenger response comprises release of inositol phosphate and the change in second messenger is an increase in the level of inositol phosphate.

123. A process for determining whether a chemical compound specifically binds to and inhibits activation of a mammalian SNORF33 receptor, which comprises separately contacting cells producing a second messenger response and expressing on their cell surface the mammalian SNORF33 receptor, wherein such cells do not normally express the mammalian SNORF33 receptor, with both the chemical compound and a second chemical compound known to activate the mammalian SNORF33 receptor, and with only the second chemical compound, under conditions suitable for activation of the mammalian SNORF33 receptor, and measuring the second messenger response in the presence of only the second chemical compound and in the presence of both the second chemical compound and the chemical compound, a smaller

change in the second messenger response in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound indicating that the chemical compound inhibits activation of the mammalian SNORF33 receptor.

124. The process of claim 123, wherein the second messenger response comprises chloride channel activation and the change in second messenger response is a smaller increase in the level of chloride current in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound.

125. The process of claim 124, wherein the second messenger response comprises change in intracellular calcium levels and the change in second messenger response is a smaller increase in the measure of intracellular calcium in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound.

126. The process of claim 123, wherein the second messenger response comprises release of inositol phosphate and the change in second messenger response is a smaller increase in the level of inositol phosphate in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound.

127. A process of any of claims 119, 120, 121, 122, 123, 124, 125, or 126, wherein the mammalian SNORF33 receptor is a human SNORF33 receptor or a rat SNORF33 receptor.

128. The process of any of claims 116, 120, 121, 122, 123, 124, 125, or 126, wherein the cell is an insect cell.

129. The process of any of claims 119, 120, 121, 122, 123, 124, 125, or 126, wherein the cell is a mammalian cell.

130. The process of claim 129, wherein the mammalian cell is nonneuronal in origin.

131. The process of claim 130, wherein the nonneuronal cell is a COS-7 cell, CHO cell, 293 human embryonic kidney cell, NIH-3T3 cell or LM(tk-) cell.

132. The process of claim 119, 120, 121, 122, 123, 124, 125, or 126, wherein the compound is not previously known to bind to a mammalian SNORF33 receptor.

133. A compound determined by the process of claim 132.

134. A composition which comprises an amount of a mammalian SNORF33 receptor agonist determined to be such by the process of claim 119, 120, 121, or 122, effective to increase activity of the mammalian SNORF33 receptor and a carrier.

135. A composition of claim 134, wherein the mammalian SNORF33 receptor agonist is not previously known.

136. A composition which comprises an amount of a mammalian SNORF33 receptor antagonist determined to be such by the process of claim 123, 124, 125, or 126, effective to reduce activity of the mammalian SNORF33 receptor and a carrier.

137. A composition of claim 136, wherein the mammalian SNORF33 receptor antagonist is not previously known.

138. A method of screening a plurality of chemical compounds not known to activate a mammalian SNORF33 receptor to identify

a compound which activates the mammalian SNORF33 receptor which comprises:

(a) contacting cells transfected with and expressing the mammalian SNORF33 receptor with the plurality of compounds not known to activate the mammalian SNORF33 receptor, under conditions permitting activation of the mammalian SNORF33 receptor;

(b) determining whether the activity of the mammalian SNORF33 receptor is increased in the presence of one or more of the compounds; and if so

(c) separately determining whether the activation of the mammalian SNORF33 receptor is increased by any compound included in the plurality of compounds, so as to thereby identify each compound which activates the mammalian SNORF33 receptor.

139. A method of claim 138, wherein the mammalian SNORF33 receptor is a human SNORF33 receptor or a rat SNORF33 receptor.

140. A method of screening a plurality of chemical compounds not known to inhibit the activation of a mammalian SNORF33 receptor to identify a compound which inhibits the activation of the mammalian SNORF33 receptor, which comprises:

(a) contacting cells transfected with and expressing the mammalian SNORF33 receptor with the plurality of compounds in the presence of a known mammalian SNORF33 receptor agonist, under conditions permitting activation of the mammalian SNORF33 receptor;

(b) determining whether the extent or amount of activation of the mammalian SNORF33 receptor is reduced in the presence of one or more of the compounds, relative to the extent or amount of activation of the mammalian SNORF33 receptor in the absence of such one or more compounds; and if so

(c) separately determining whether each such compound inhibits activation of the mammalian SNORF33 receptor for each compound included in the plurality of compounds, so as to thereby identify any compound included in such plurality of compounds which inhibits the activation of the mammalian SNORF33 receptor.

141. A method of claim 140, wherein the mammalian SNORF33 receptor is a human SNORF33 receptor or rat SNORF33 receptor.

142. A method of any of claims 138, 139, 140, 141, wherein the cell is a mammalian cell.

143. A method of claim 142, wherein the mammalian cell is non-neuronal in origin.

144. The method of claim 143, wherein the non-neuronal cell is a COS-7 cell, a 293 human embryonic kidney cell, a LM(tk-) cell or an NIH-3T3 cell.

145. A composition comprising a compound identified by the method of claim 138 or 139 in an amount effective to increase mammalian SNORF33 receptor activity and a carrier.

146. A composition comprising a compound identified by the method of claim 140 or 141 in an amount effective to decrease mammalian SNORF33 receptor activity and a carrier.

147. A method of treating an abnormality in a subject wherein the abnormality is alleviated by increasing the activity of a mammalian SNORF33 receptor which comprises administering to the subject a compound which is a mammalian SNORF33 receptor agonist in an amount effective to treat the abnormality.

148. A method of treating an abnormality in a subject wherein the abnormality is alleviated by decreasing the activity of a mammalian SNORF33 receptor which comprises administering to the subject a compound which is a mammalian SNORF33 receptor antagonist in an amount effective to treat the abnormality.

149. A process for making a composition of matter which specifically binds to a mammalian SNORF33 receptor which comprises identifying a chemical compound using the process of any of claims 58, 59, 77, 78, 87 or 88 and then synthesizing the chemical compound or a novel structural and functional analog or homolog thereof.

150. The process of claims 149, wherein the mammalian SNORF33 receptor is a human SNORF33 receptor or a rat SNORF33 receptor.

151. A process for making a composition of matter which specifically binds to a mammalian SNORF33 receptor which comprises identifying a chemical compound using the process of any of claims 112, 119, or 138 and then synthesizing the chemical compound or a novel structural and functional analog or homolog thereof.

152. The process of claim 151, wherein the mammalian SNORF33 receptor is a human SNORF33 receptor or a rat SNORF33

receptor.

153. A process for making a composition of matter which specifically binds to a mammalian SNORF33 receptor which comprises identifying a chemical compound using the process of any of claims 113, 123 or 140 and then synthesizing the chemical compound or a novel structural and functional analog or homolog thereof.

154. The process of claim 153, wherein the mammalian SNORF33 receptor is a human SNORF33 receptor or a rat SNORF33 receptor.

155. A process for preparing a composition which comprises admixing a carrier and a pharmaceutically effective amount of a chemical compound identified by the process of any of claims 58, 59, 77, 78, 87 or 88 or a novel structural and functional analog or homolog thereof.

156. The process of claim 155, wherein the mammalian SNORF33 receptor is a human SNORF33 receptor or a rat SNORF33 receptor.

157. A process for preparing a composition which comprises admixing a carrier and a pharmaceutically effective amount of a chemical compound identified by the process of any of claims 112, 119, or 138 or a novel structural and functional analog or homolog thereof.

158. The process of claim 157, wherein the mammalian SNORF33 receptor is a human SNORF33 receptor or a rat SNORF33 receptor.

159. A process for preparing a composition which comprises admixing a carrier and a pharmaceutically effective amount

of a chemical compound identified by the process of any of claims 113, 123 or 140 or a novel structural and functional analog or homolog thereof.

160. The process of claim 159, wherein the mammalian SNORF33 receptor is a human SNORF33 receptor or a rat SNORF33 receptor.

DNA ENCODING SNORF33 RECEPTOR

Abstract of the Disclosure

This invention provides isolated nucleic acids encoding mammalian SNORF33 receptors, purified mammalian SNORF33 receptors, vectors comprising nucleic acid encoding mammalian SNORF33 receptors, cells comprising such vectors, antibodies directed to mammalian SNORF33 receptors, nucleic acid probes useful for detecting nucleic acid encoding mammalian SNORF33 receptors, antisense oligonucleotides complementary to unique sequences of nucleic acid encoding mammalian SNORF33 receptors, transgenic, nonhuman animals which express DNA encoding normal or mutant mammalian SNORF33 receptors, methods of isolating mammalian SNORF33 receptors, methods of treating an abnormality that is linked to the activity of the mammalian SNORF33 receptors, as well as methods of determining binding of compounds to mammalian SNORF33 receptors, methods of identifying agonists and antagonists of SNORF33 receptors, and agonists and antagonists so identified.

FIGURE 1

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1    ACTGTGGACTTTCTTCTGGGGTGTCTGGTCATGCCTTACAGTATGGTGAGATCTGCTGAG   60
61   CACTGTTGGTATTTTGGAGAAGTCTTCTGTAAAATTCACACAAGCACCGACATTATGCTG   120
121  AGCTCAGCCTCCATTTTCCATTTGTCTTTCATCTCCATTGACCGCTACTATGCTGTGTGT   180
181  GATCCACTGAGATATAAAGCCAAGATGAATATCTTGGTTATTTGTGTGATGATCTTCATT   240
241  AGTTGGAGTGTCCCTGCTGTTTTTGCATTTGGAATGATCTTTCTGGAGCTAAACTTCAAA   300
301  GGCGCTGAAGAGATATATTACAAACATGTTCACTGCAGAGGAGGTTGCTCTGTCTTCTTT   360
361  AGCAAAATATCTGGGGTACTGACCTTTATGACTTCTTTTTATATACCTGGATCTATTATG   420
421  TTATGTGTCTATTACAGAATATATCTTATCGCTAAAGAACAGGCAAGATTAATTAGTGAT   480
481  GCCAATCAGAAGCTCCAAATTGGATTGGAAATGAAAAATGGAATTTCACAAAGCAAAGAA   540
541  AGGAAAGCTGTGAAGACATTGGGGATTGTGATG                              573
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FIGURE 2

1	T	V	D	F	L	L	G	C	L	V	M	P	Y	S	M	V	R	S	A	E	20
21	H	C	W	Y	F	G	E	V	F	C	K	I	H	T	S	T	D	I	M	L	40
41	S	S	A	S	I	F	H	L	S	F	I	S	I	D	R	Y	Y	A	V	C	60
61	D	P	L	R	Y	K	A	K	M	N	I	L	V	I	C	V	M	I	F	I	80
81	S	W	S	V	P	A	V	F	A	F	G	M	I	F	L	E	L	N	F	K	100
101	G	A	E	E	I	Y	Y	K	H	V	H	C	R	G	G	C	S	V	F	F	120
121	S	K	I	S	G	V	L	T	F	M	T	S	F	Y	I	P	G	S	I	M	140
141	L	C	V	Y	Y	R	I	Y	L	I	A	K	E	Q	A	R	L	I	S	D	160
161	A	N	Q	K	L	Q	I	G	L	E	M	K	N	G	I	S	Q	S	K	E	180
181	R	K	A	V	K	T	L	G	I	V	M										191

FIGURE 3A

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1    ATTGCTCGACAGCCAAAGGGACAGAGCAGCCTGTGTTTAGTTCTCTGTAGTGATGCATCT  60
61   TTGCCACAATAGCGCGAATATTTCCCACACGAACAGCAACTGGTCAAGGGATGTCCGTGC  120
121  TTCGCTGTACAGCTTAATATCACTCATAATTCTAACCACTCTGGTTGGCAACTTAATAGT  180
181  AATCATTTCGATATCCCACTTCAAGCAACTTCACACGCCCACAAATTGGCTCCTTCATTC  240
241  CATGGCCGTTGTCGACTTTCTGCTGGGCTGTCTGGTCATGCCCTACAGCATGGTGAGAAC  300
301  AGTTGAGCACTGCTGGTACTTTGGGGAACTCTTCTGCAAACTTCACACCAGCACTGATAT  360
361  CATGCTGAGCTCGGCATCCATTCTCCACCTAGCCTTCATTTCCATTGACCGCTACTATGC  420
421  TGTGTGCGACCCTTTAAGATACAAAGCCAAGATCAATCTCGCCGCCATTTTTGTGATGAT  480
481  CCTCATTAGCTGGAGCCTTCCTGCTGTTTTTGCATTTGGGATGATCTTCCTGGAGCTGAA  540
541  CTTAGAAGGAGTTGAGGAGCTGTATCACAATCAGGTCTTCTGCCTGCGCGGCTGTTTTCC  600
601  CTTCTTCAGTAAAGTATCTGGGGTACTGGCATTCATGACGTCTTTCTATATACCTGGATC  660
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FIGURE 3B

661	TGTTATGTTATTTGTTTACTATAGAATATATTTCATAGCTAAAGGACAAGCAAGGTCAAT	720
721	TAATCGTGCAAATCTTCAAGTTGGATTGGAAGGGGAAAGCAGAGCGCCACAAAGCAAGGA	780
781	AACAAAAGCCGCGAAAACCTTAGGGATCATGGTGGGCGTTTTCCTCCTGTGCTGGTGCCC	840
841	GTTCTTTTTCTGCATGGTCCTGGACCCTTTCCTGGGCTATGTTATCCCACCCACTCTGAA	900
901	TGACACACTGAATTGGTTTGGGTACCTGAACTCTGCCTTCAACCCGATGGTTTATGCCTT	960
961	TTTCTATCCCTGGTTCAGAAGAGCGTTGAAGATGGTTCTCTTCGGTAAAATTTTCCAAAA	1020
1021	AGATTCATCTAGGTCTAAGTTATTTTTGTAACGCAATCCATGAAACCAGTATATTTTGTA	1080
1081	GTTCTTAAGAGCAGTTGGTGA	1101

FIGURE 4A

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1    M H L C H N S A N I S H T N S N W S R D   20
21   V R A S L Y S L I S L I I L T T L V G N   40
41   L I V I I S I S H F K Q L H T P T N W L   60
61   L H S M A V V D F L L G C L V M P Y S M   80
81   V R T V E H C W Y F G E L F C K L H T S   100
101  T D I M L S S A S I L H L A F I S I D R   120
121  Y Y A V C D P L R Y K A K I N L A A I F   140
141  V M I L I S W S L P A V F A F G M I F L   160
161  E L N L E G V E E L Y H N Q V F C L R G   180
181  C F P P F F S K V S G V L A F M T S F Y I  200
201  P G S V M L F V Y Y R I Y F I A K G Q A   220
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FIGURE 4B

221 R S I N R A N L Q V G L E G E S R A P Q 240
241 S K E T K A A K T L G I M V G V F L L C 260
261 W C P F F F C M V L D P F L G Y V I P P 280
281 T L N D T L N W F G Y L N S A F N P M V 300
301 Y A F F Y P W F R R A L K M V L F G K I 320
321 F Q K D S S R S K L F L 332

FIGURE 5A

1 TCAGGAATGATGCCCTTTTGCCACAATATAATTAATATTTCCTGTGTGAAAAACAACTGG 60

61 TCAAATGATGTCCGTGCTTCCCTGTACAGTTTAATGGTGCTCATAATTCTGACCACACTC 120

121 GTTGGCAATCTGATAGTTATTGTTTCTATATCACACTTCAAACAACTTCATACCCCAACA 180

181 AATTGGCTCATTCATTCCATGGCCACTGTGGACTTTCTTCTGGGGTGTCTGGTCATGCCT 240

241 TACAGTATGGTGAGATCTGCTGAGCACTGTTGGTATTTTGGAGAAGTCTTCTGTAAAATT 300

301 CACACAAGCACCGACATTATGCTGAGCTCAGCCTCCATTTTCCATTTGTCTTTCATCTCC 360

361 ATTGACCGCTACTATGCTGTGTGTGATCCACTGAGATATAAAGCCAAGATGAATATCTTG 420

421 GTTATTTGTGTGATGATCTTCATTAGTTGGAGTGTCCCTGCTGTTTTTGCATTTGGAATG 480

481 ATCTTTCTGGAGCTAAACTTCAAAGGCGCTGAAGAGATATATTACAAACATGTTCACTGC 540

541 AGAGGAGGTTGCTCTGTCTTCTTTAGCAAAATATCTGGGGTACTGACCTTTATGACTTCT 600

601 TTTTATATACCTGGATCTATTATGTTATGTGTCTATTACAGAATATATCTTATCGCTAAA 660

FIGURE 5B

661 GAACAGGCAAGATTAATTAGTGATGCCAATCAGAAGCTCCAAATTGGATTGGAAATGAAA 720

721 AATGGAATTTCACAAAGCAAAGAAAGGAAAGCTGTGAAGACATTGGGGATTGTGATGGGA 780

781 GTTTTCCTAATATGCTGGTGCCCTTTCTTTATCTGTACAGTCATGGACCCTTTTCTTCAC 840

841 TACATTATTCCACCTACTTTGAATGATGTGTTGATTTGGTTTGGCTACTTGAACTCTACA 900

901 TTTAATCCAATGGTTTATGCATTTTTCTATCCTTGGTTTAGAAAAGCACTGAAGATGATG 960

961 CTGTTTGGTAAAATTTTCCAAAAAGATTCATCCAGGTGTAAATTATTTTTGGAATTGAGT 1020

1021 TCATAGAATTATTATATT 1038

FIGURE 6A

1	M	M	P	F	C	H	N	I	I	N	I	S	C	V	K	N	N	W	S	N	20
21	D	V	R	A	S	L	Y	S	L	M	V	L	I	I	L	T	T	L	V	G	40
41	N	L	I	V	I	V	S	I	S	H	F	K	Q	L	H	T	P	T	N	W	60
61	L	I	H	S	M	A	T	V	D	F	L	L	G	C	L	V	M	P	Y	S	80
81	M	V	R	S	A	E	H	C	W	Y	F	G	E	V	F	C	K	I	H	T	100
101	S	T	D	I	M	L	S	S	A	S	I	F	H	L	S	F	I	S	I	D	120
121	R	Y	Y	A	V	C	D	P	L	R	Y	K	A	K	M	N	I	L	V	I	140
141	C	V	M	I	F	I	S	W	S	V	P	A	V	F	A	F	G	M	I	F	160
161	L	E	L	N	F	K	G	A	E	E	I	Y	Y	K	H	V	H	C	R	G	180
181	G	C	S	V	F	F	S	K	I	S	G	V	L	T	F	M	T	S	F	Y	200
201	I	F	G	S	I	M	L	C	V	Y	Y	R	I	Y	L	I	A	K	E	Q	220

FIGURE 6B

```
221   A R L I S D A N Q K L Q I G L E M K N G   240
241   I S Q S K E R K A V K T L G I V M G V F   260
261   L I C W C P F F I C T V M D P F L H Y I   280
281   I P P T L N D V L I W F G Y L N S T F N   300
301   P M V Y A F F Y P W F R K A L K M M L F   320
321   G K I F Q K D S S R C K L F L E L S S *   340
```

FIGURE 7A

```
Rat SNORF33    1 .MHLCHNSANISHTNSNWSRDVRASLYSLISLIILTTLVGNLIVIISISH  49
                  |  |||  |||   .||| |||||||||. ||||||||||||||:||||
Hum SNORF33    1 MMPFCHNIINISCVKNNWSNDVRASLYSLMVLIILTTLVGNLIVIVSISH  50

Rat SNORF33   50 FKQLHTPTNWLLHSMAVVDFLLGCLVMPYSMVRTVEHCWYFGELFCKLHT  99
                 |||||||||||:|||| ||||||||||||||||. ||||||||.|||:||
Hum SNORF33   51 FKQLHTPTNWLIHSMATVDFLLGCLVMPYSMVRSAEHCWYFGEVFCKIHT 100

Rat SNORF33  100 STDIMLSSASILHLAFISIDRYYAVCDPLRYKAKINLAAIFVMILISWSL 149
                 ||||||||||| ||.|||||||||||||||||||.|:  | ||| ||||.
Hum SNORF33  101 STDIMLSSASIFHLSFISIDRYYAVCDPLRYKAKMNILVICVMIFISWSV 150

Rat SNORF33  150 PAVFAFGMIFLELNLEGVEELYHNQVFCLRGCFPFFSKVSGVLAFMTSFY 199
                 |||||||||||||| .| ||:|:  | |  ||  ||||:|||| ||||||
Hum SNORF33  151 PAVFAFGMIFLELNFKGAEEIYYKHVHCRGGCSVFFSKISGVLTFMTSFY 200

Rat SNORF33  200 IPGSVMLFVYYRIYFIAKGQARSINRAN..LQVGLEGESRAPQSKETKAA 247
                 ||||:|| |||||| ||| ||| |. ||  ||:||| ..   |||| ||
Hum SNORF33  201 IPGSIMLCVYYRIYLIAKEQARLISDANQKLQIGLEMKNGISQSKERKAV 250
```

FIGURE 7B

```
Rat SNORF33  248 KTLGIMVGVFLLCWCPFFFCMVLDPFLGYVIPPTLNDTLNWFGYLNSAFN 297
                 |||||..||||:||||| | |:|||| |:||||||| | ||||||| ||
Hum SNORF33  251 KTLGIVMGVFLICWCPFFICTVMDPFLHYIIPPTLNDVLIWFGYLNSTFN 300

Rat SNORF33  298 PMVYAFFYPWFRRALKMVLFGKIFQKDSSRSKLFL....*            333
                 ||||||||||||:||||.||||||||||||| ||||
Hum SNORF33  301 PMVYAFFYPWFRKALKMMLFGKIFQKDSSRCKLFLELSS*            340
```

FIGURE 8

Basal cAMP Levels in Mock- and rat SNORF33-Transfected Cos-7 cells

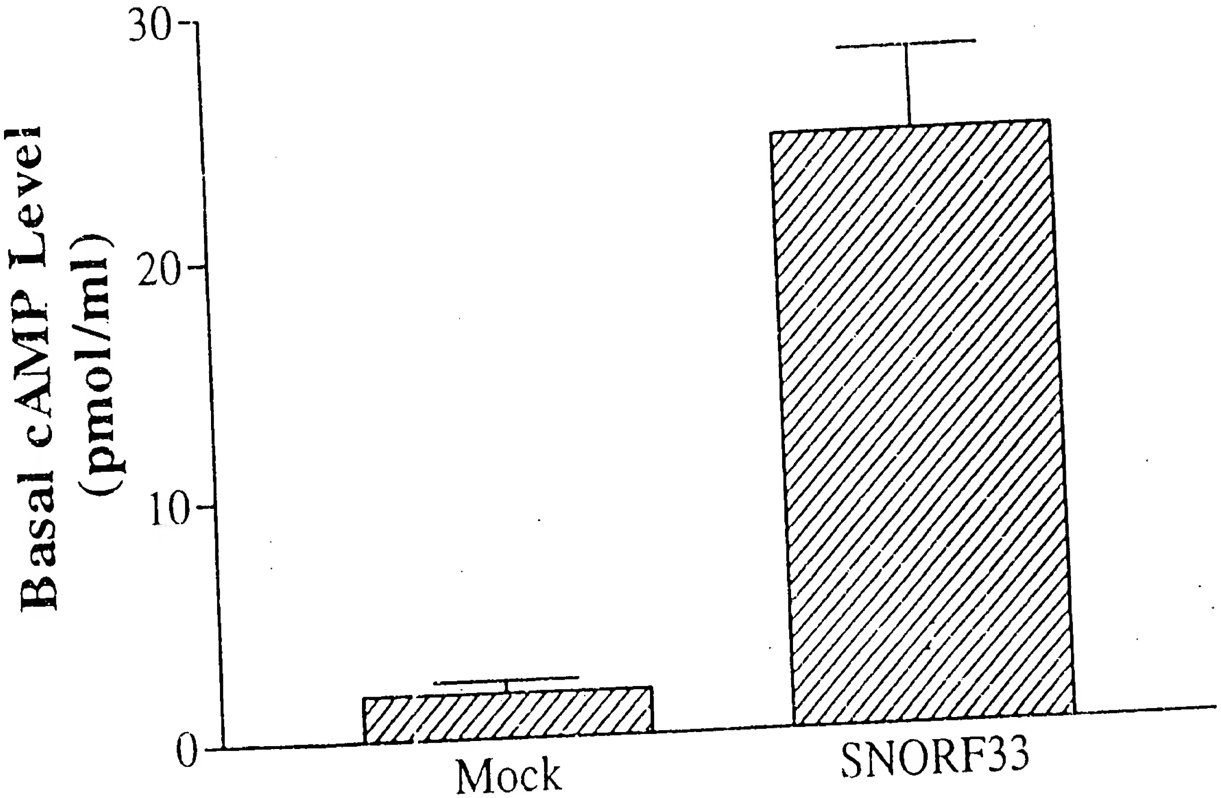


FIGURE 9

Agonist-Mediated Increase in Intracellular cAMP Levels in Mock- and rat SNORF33-Transfected Cos-7 Cells

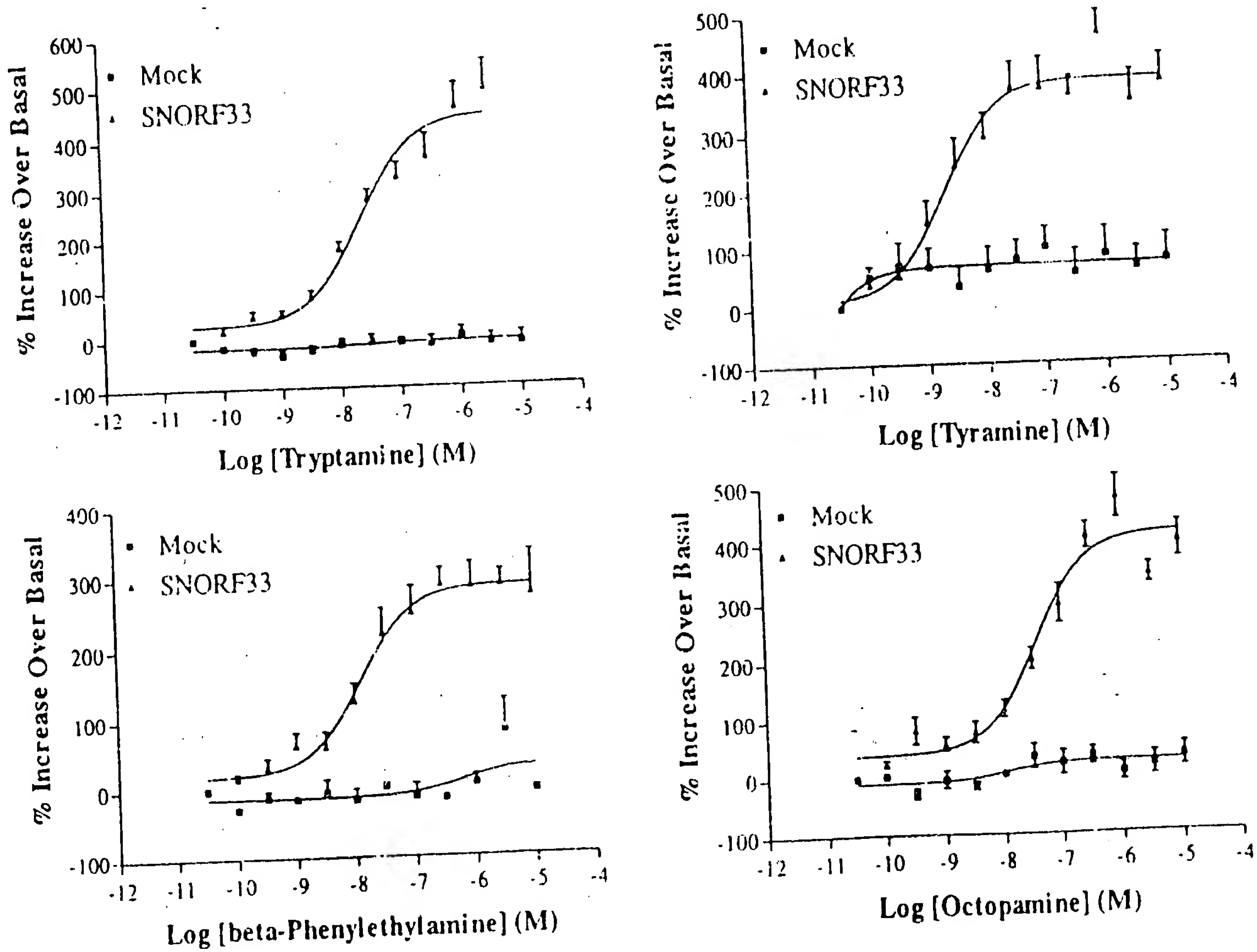


FIGURE 10

Agonist-Mediated Increase in Intracellular cAMP Levels in Mock- and rat SNORF33-Transfected Cos-7 Cells

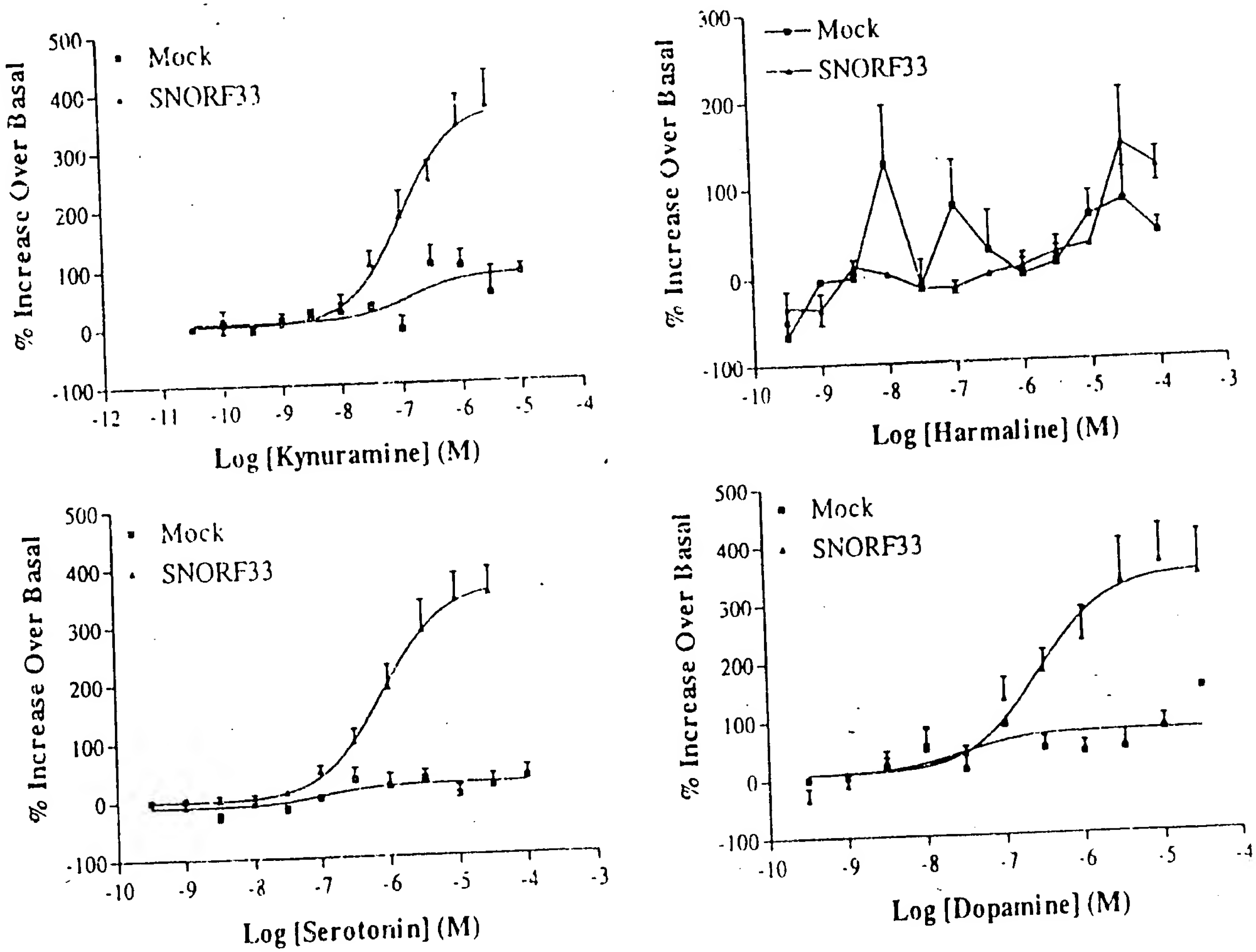


Figure 11A

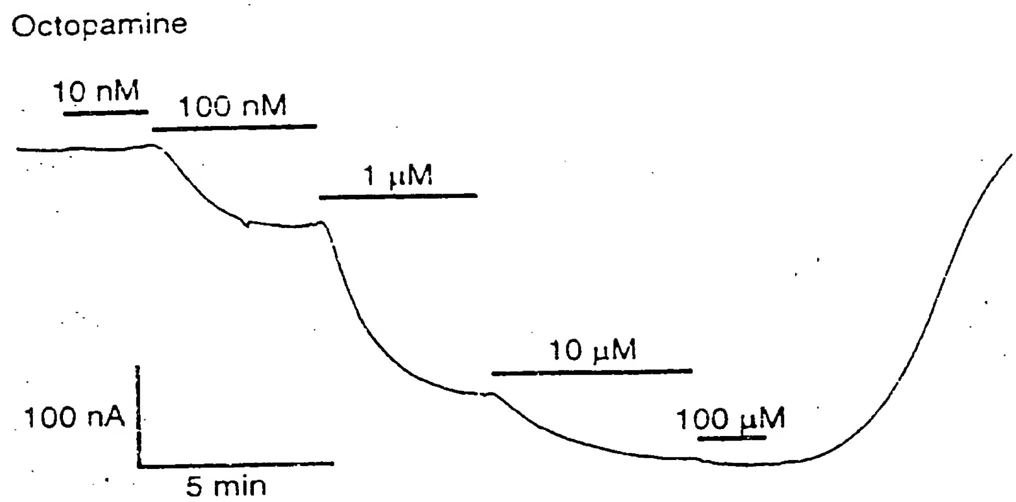


Figure 11B

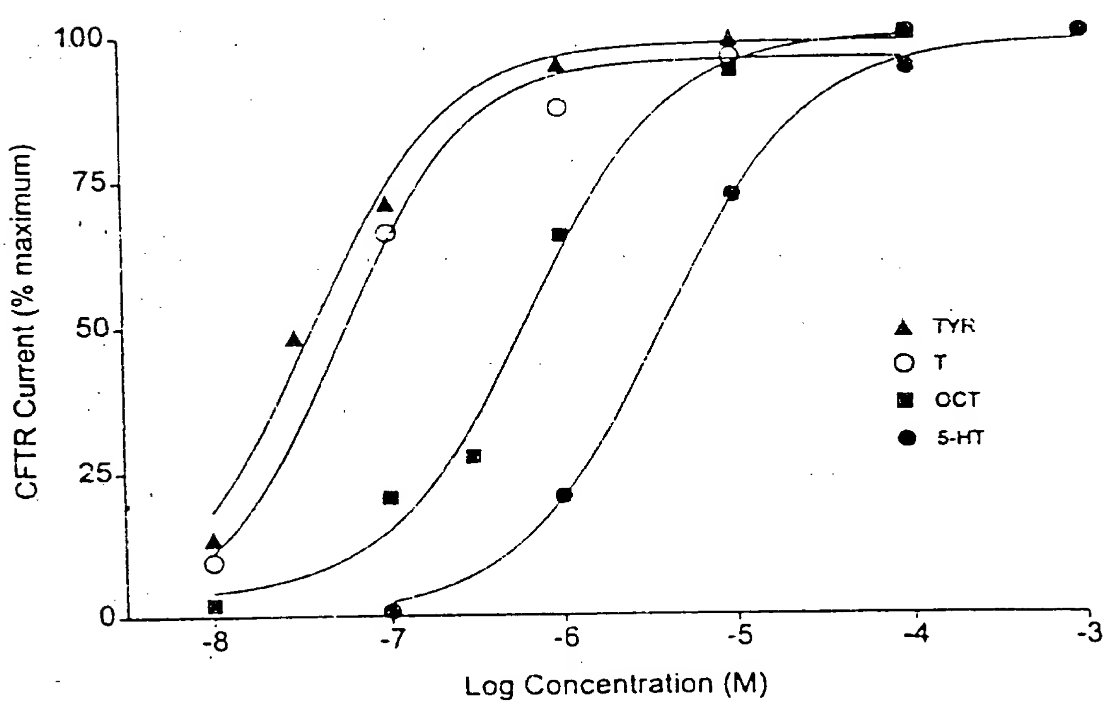
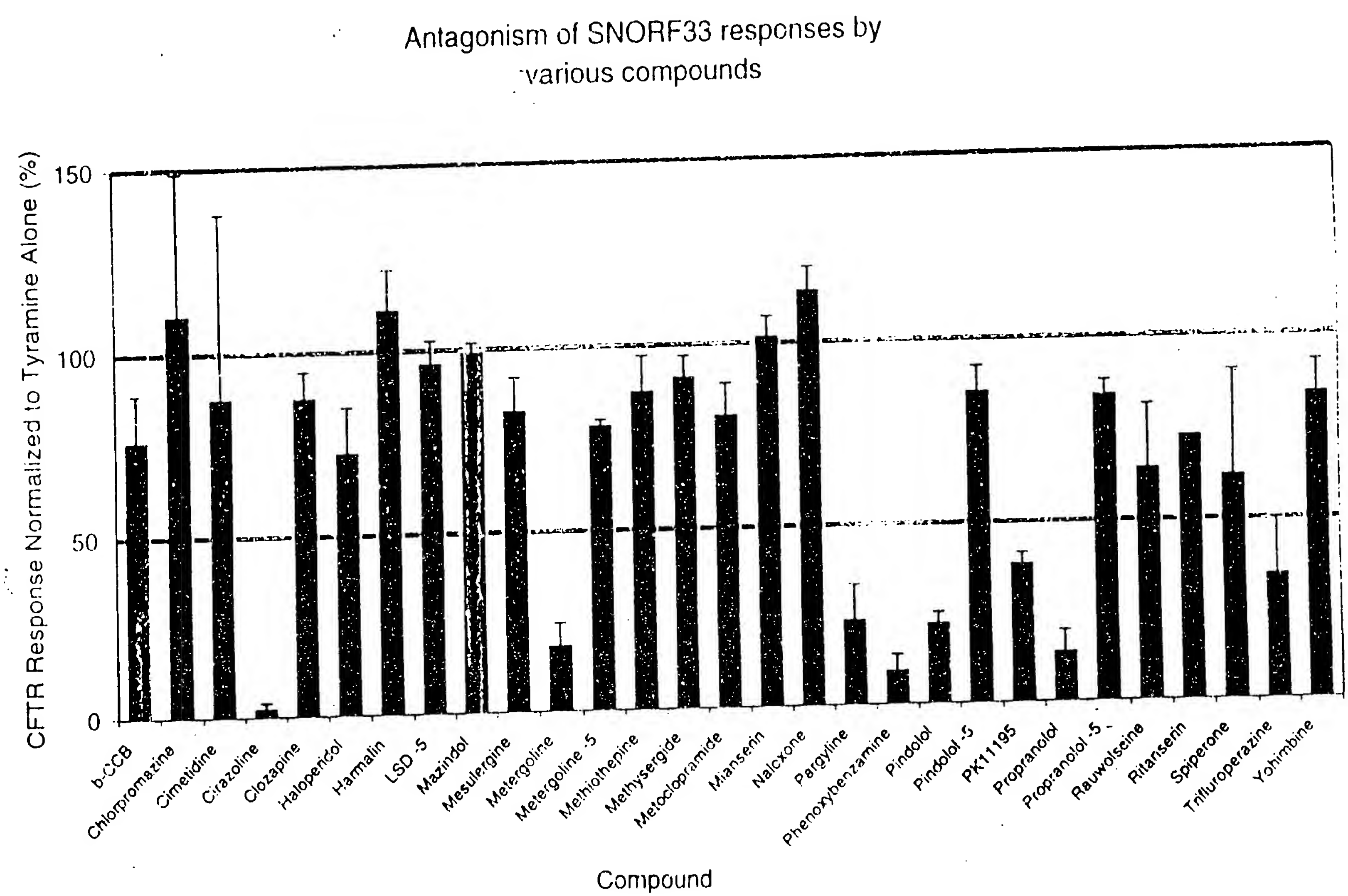


Figure 12



Antagonism of SNORF33 responses by various compounds
CFTR Response Normalized to Tyramine Alone (%)
150
100
50
0
b-CCB
Chlorpromazine
Cimetidine
Cirazoline
Clozapine
Halopendol
Harmalin
LSD-5
Mazindol
Mesulergine
Metergoline
Metergoline-5
Methiothepine
Methysergide
Metoclopramide
Mianserin
Nalcxone
Pargyline
Phenoxybenzamine
Pindolol
Pindolol-5
PK11195
Propranolol
Propranolol-5
Rauwolscine
Ritanserin
Spiperone
Trifluoperazine
Yohimbine
Compound

Figure 13

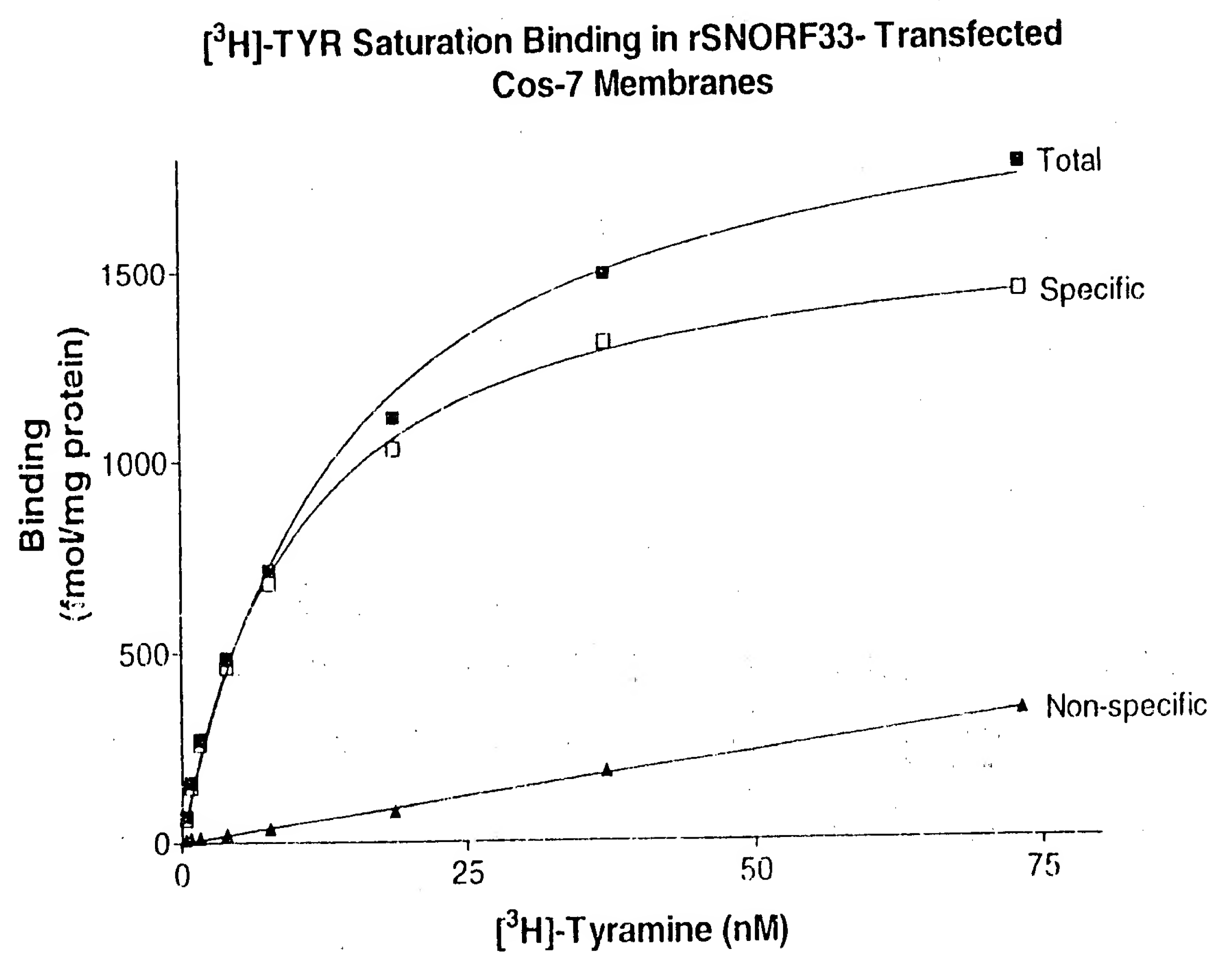

[3H]-TYR Saturation Binding in rSNORF33- Transfected
Cos-7 Membranes
Total
Specific
Non-specific
Binding
(fmol/mg protein)
1500
1000
500
0
0
25
50
75
[3H]-Tyramine (nM)

Figure 14

Displacement of Specific [^{3}H]-TYR Binding in rSNORF33-Transfected Cos-7 Membranes

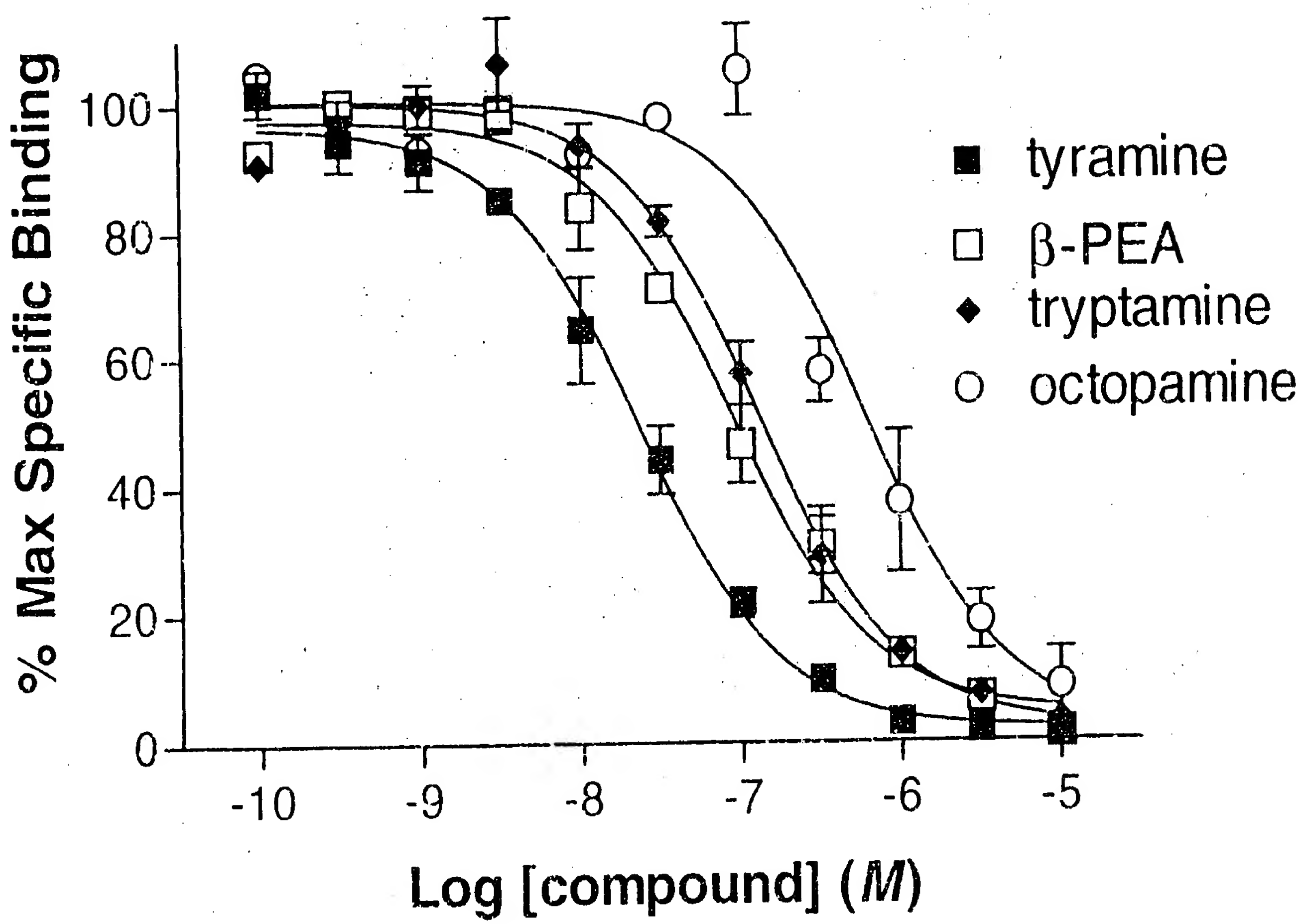


Figure 15

$[^3H]$-T (20 nM) Binding in rSNORF33- and Mock-Transfected Cos-7 Membranes

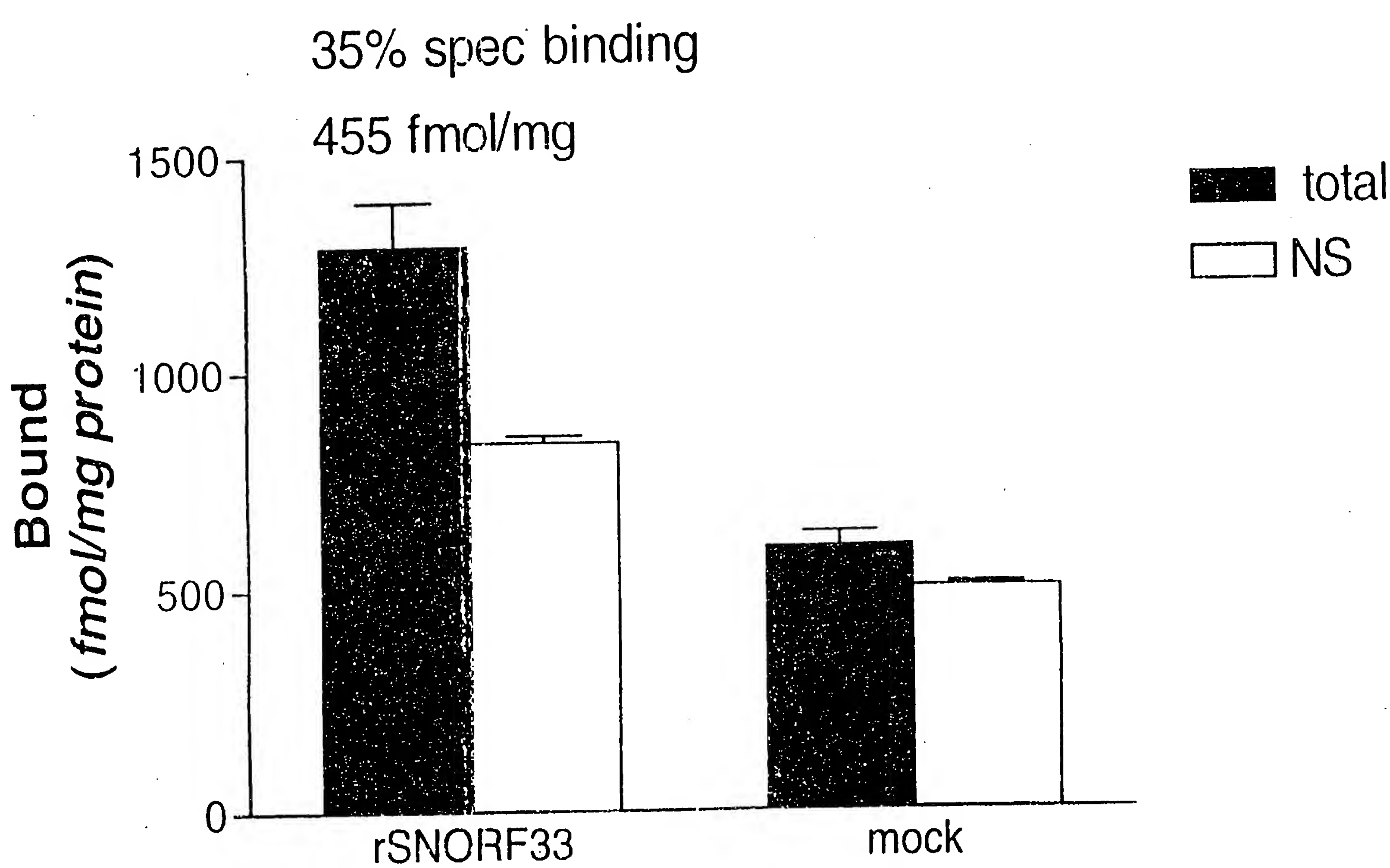


Figure 16

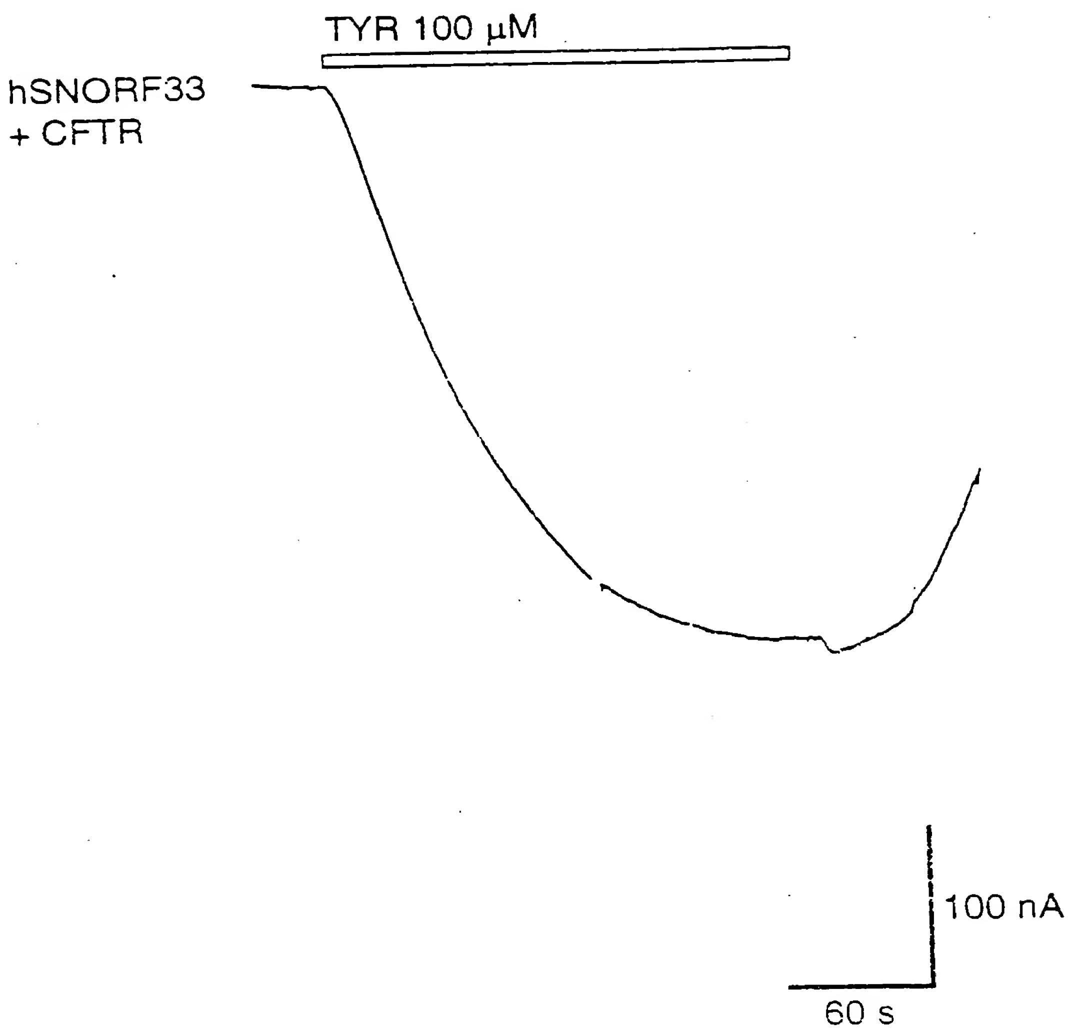


Figure 17

```
1    GGTACTGGCGTTCATGACTTCCTTCTATATACCTGGATCTGTTATGTTATTTGTTTACTA   60
61   TAGGATATATTTCATAGCTAAAGGACAAGCAAGGTCAATCAATCGTACGAATGTTCAAGT   120
121  TGGATTGGAAGGGAAAAGCCAAGCACCACAAAGCAAGGAAACAAAAGCCGCGAAGACCTT   180
181  AGGGATCATGGTGGGCGTTTTCCTCGTATGCTGGTGCCCGTTCTTTCTCTGCACGGTCCT   240
241  GGACCCTTTCCT                                                   252
```

Figure 18

1	V L A F M T S F Y I P G S V M L F V Y Y	20
21	R I Y F I A K G Q A R S I N R T N V Q V	40
41	G L E G K S Q A P Q S K E T K A A K T L	60
61	G I M V G V F L V C W C P F F L C T V L	80
81	D P F	83